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14. ABSTRACT
The 2013 Annual Meeting of Institute of Biological Engineering (IBE) was successfully held at the Embassy Suite Research Triangle Hotel, Cary, North Carolina, March 7-9, 2013. The meeting had 174 registered attendees from academia, industry, and government agencies across the State and a few internationals, with 107 podium presentations and 39 poster presentations. The meeting had 4 General Sessions for broad participation of all attendees, these sessions included Frontiers in Biological Engineering, Funding Opportunities for Biological Engineering, Biobusiness Nexus for moving technology for Translation, and Bioethics. It also held 11 scientific

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Report Title

Final Report: The Institute of Biological Engineering 2013 Annual Conference

ABSTRACT

The 2013 Annual Meeting of Institute of Biological Engineering (IBE) was successfully held at the Embassy Suite Research Triangle Hotel, Cary, North Carolina, March 7-9, 2013. The meeting had 174 registered attendees from academia, industry, and government agencies across the State and a few internationals, with 107 podium presentations and 39 poster presentations. The meeting had 4 General Sessions for broad participation of all attendees, these sessions included Frontiers in Biological Engineering, Funding Opportunities for Biological Engineering, Biobusiness Nexus for moving technology for Translation, and Bioethisc. It also held 11 scientific sessions in different areas which included Biomaterials & Structures, Bioenergy, Sensors & Biosensors, Nanomaterials & Nanosystems, Ecological and Environmental Engineering, Metabolic Pathway Engineering, Tissue and Cellular Engineering, and Synthetic Biology.

The Army Research Office (ARO) supported the travel expenses for the keynote and invited speakers and partial meeting expenses for printing meeting materials. It was a successful and meaningful meeting in North Carolina that provided opportunities for national and international attendees and local attendees to share new information and to build collaborative networks.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received	Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Post Doctorates

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Faculty Supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

Please see attachments for The Meeting program and proceeding.

Technology Transfer

Final Report: The Institute of Biological Engineering 2013 Annual Conference

Proposal Number:	63691LSCF
Agreement Number:	W911NF1310049
Proposal Title:	The Institute of Biological Engineering 2013 Annual Conference
Report Period Begin Date:	03/01/2013
Report Period End Date:	12/31/2013

Abstract

The 2013 Annual Meeting of Institute of Biological Engineering (IBE) was successfully held at the Embassy Suite Research Triangle Hotel, Cary, North Carolina, March 7-9, 2013. The meeting had 174 registered attendees from academia, industry, and government agencies across the State and a few internationals, with 107 podium presentations and 39 poster presentations. The meeting had 4 General Sessions for broad participation of all attendees, which included Frontiers in Biological Engineering, Funding Opportunities for Biological Engineering, Biobusiness Nexus for moving technology for Translation, and Bioethisc. It also held 11 scientific sessions in different areas which included Biomaterials & Structures, Bioenergy, Sensors & Biosensors, Nanomaterials & Nanosystems, Ecological and Environmental Engineering, Metabolic Pathway Engineering, Tissue and Cellular Engineering, and Synthetic Biology.

The Army Research Office (ARO) supported the travel expenses for the keynote and invited speakers and partial meeting expenses for printing meeting materials. It was a successful and meaningful meeting in North Carolina that provided opportunities for national and international attendees and local attendees to share new information and to build collaborative networks.

Keynote Speaker:

Dr. Randolph V. Lewis, USTAR Professor of Biology, Utah State University.

Presentation: Spider Silk: Developing an Ancient Biomaterial for the Future

Invited Speakers:

Dr. Erik Reimhult (supported by MRSEC)
University Professor, Dept. of Nanobiotechnology
University of Natural Resources & Life Sciences, Vienna, Austria
Presentation: Micro and nanostructured lipid membranes for sensing and cell manipulation

Dr. Suzie Pun (Supported by the Grant)
Robert J. Rushmer Associate Professor of Bioengineering
University of Washington

Presentation: Peptide-Based materials for Drug Delivery

Dr. Ya-Ping Sun (Supported by the Grant**)**
Frank Henry Leslie Professor of Chemistry,
Clemson University
Presentation: Fluorescent Carbon Dots for Bioimaging and Beyond

Dr. Gabriel Lopez (Local)
Professor of Biomedical Engineering and Mechanical Engineering and Materials Science
Duke University
Presentation: Acoustic Microfluidics and New Biofunctional Colloids for Bioanalytical Applications

Dr. D. Marshall Porterfield (Federal Government)
Professor of Biological Engineering and Biomedical Engineering
Purdue University
Division Director, NASA Life and Physical Sciences Division
Presentation: Engineered Biological Systems for Long Duration Human Space Exploration

SCHEDULE OF PRESENTATIONS *(Updated March 5, 2013)*

THURSDAY AFTERNOON, MARCH 7, 2013

1:00 pm – 5:00 pm **Registration**

3:00 pm – 5:00 pm **IBE Executive Council Meeting**

5:00 pm – 6:00 pm **Welcome Reception**

6:00 pm **Dinner (on your own)**

FRIDAY MORNING, MARCH 8, 2013

7:00 am – 8:00 am **Breakfast/IBE Committee Meetings**
Organizers: Dr. Czarena Crofcheck and IBE Committee Chairs

8:00 am – 9:20 am **Opening Remarks/Keynote Address**

8:00 am **Opening Remarks**
Dr. Czarena Crofcheck, President of IBE 2013

8:10 am **Keynote Address:** *Spider Silk: Developing an Ancient Biomaterial for the Future*
Dr. Randolph V. Lewis
USTAR Professor of Biology
Utah State University

9:20 am – 9:30 am **Break**

9:30 am – 12:00 pm **Four Concurrent Sessions**

I-A: SENSORS & BIOSENSORS 1
Chairs: Dr. Stefan Zauscher, Duke University; Dr. Amani Wan Salim, Purdue University

9:30 am Engineering Whispering Gallery Mode Optical Biosensors for Environmental Monitoring Sensors and Biosensors
Heather K. Hunt, Matthew T. Bernards, Carol E. Soteropoulos, Emily O'Brien, University of Missouri

9:45 am Enzyme-based Biosensors for Studying Methanogenic Biofilm Physiology
Stephanie Burrs, Diana Vanegas, Masashige Taguchi, Prachee Chaturvedi, University of Florida

10:00 am Micro and nanostructured lipid membranes for sensing and cell manipulation
Invited Speaker: Erik Reimhult, University Professor, Dept. of Nanobiotechnology
University of Natural Resources & Life Sciences, Vienna, Austria

10:30 am Break

10:45 am Graphene Bio-Nanosensing
Yue Cui, Utah State University

11:00 am Comparative studies on nanomaterial platforms for biosensors to monitor meat quality
Diana Vanegas, Stephanie Burrs, Prachee Chaturvedi, Masashige Taguchi, Katelyn Ward, Arthur Teixeira, Eric McLamore, University of Florida

11:15 am Complex supported lipid bilayers with high cholesterol content formed by helical eptide-induced vesicle fusion
Gregory Hardy, Rahul Nayak, Munir Alam, Joe Shapter, Stefan Zauscher, Duke University

11:30 am Non-destructive optical oxygen sensing using PtTFPP (Platinum Tetrakis Pentafluorophenyl Porphyrin) on long term shelf life study for food packaging
Kul Inn, Wan W. Amani Wan Salim, Purdue University; D. Marshall Porterfield, Purdue University, NASA Headquarters

11:45 am Directed functionalization strategies for high-resolution optical fiber based biosensors
Rajtarun Madangopal, Matthew C. Stensberg, Leyla Nesrin Kahyaoglu, Jenna L. Rickus, Purdue University; D. Marshall Porterfield, Purdue University, NASA Headquarters

I-B: BIOMATERIALS & STRUCTURES 1
Chair: Dr. Darlene Taylor, North Carolina Central University

9:30 am Peptide-Based materials for Drug Delivery
Invited Speaker: Dr. Suzie Pun
Robert J. Rushmer Associate Professor of Bioengineering
University of Washington

10:00 am Nano-engineered Polymer Coatings and Capsules for Controlled Drug Delivery and Cell Transplantation
Eugenia Kharlampieva, University of Alabama - Birmingham

10:15 am Exploiting Softlithographic Techniques to Make Particles for Applications in Life and Material Sciences
Charles J. Bowerman , Kevin Chu, Kevin Reuter, Jillian Perry, Marc Kai, Tammy Shen, Cathy Fromen, Dominica Wong, Yapei Wang, Chris Luft, Joseph M. DeSimone, University of North Carolina at Chapel Hill

10:30 am Break

10:45 am Electrospun Nanofibers as Functional Biomaterials
Christopher A. Bonino, Carl D. Saquing, Saad A. Khan, North Carolina State University

11:00 am MicroSCALE screening reveals genetic modifiers of therapeutic response in melanoma
Kris C. Wood, Duke University

11:15 am Substrate Topography Influences the Functional Neurons Produced by Direct Reprogramming of Fibroblasts
Karina Kulangara, Andrew F. Adler, Hong Wang, Malathi Chellappan, Ellen Hammett, Ryohei Yasuda, Kam W. Leong, Duke University

11:30 am Multifunctional thermo-responsive polymer brushes with dynamic nanotopology for attachment, killing and release of bacteria
Qian Yu, Janghwan Cho, Phanindhar Shivapooja, NSF Research Triangle Materials Research Science and Engineering Center, Department of Biomedical Engineering, Duke University; Gabriel P. Lopez, NSF Research Triangle Materials Research Science and Engineering Center, Department of Biomedical Engineering, Duke University, Department of Mechanical Engineering and Materials Science, Duke University

11:45 am A pH-sensitive starch composite
Yuzhi Deng, Pennsylvania State University

I-C: BIOENERGY: BIOCHEMICAL CONVERSIONS
Chairs: Dr. Yanna Liang, Southern Illinois University; Dr. Pratap Pullamanapalil, University of Florida

9:30 am Advances in the analysis of sweet sorghum composition for bioprocess development
Matthew B. Whitfield, Mari S. Chinn, Matthew W. Veal, North Carolina State University

9:45 am Correlation between Lignin Monomers Recovery and Fermentable Sugars Generation from Miscanthus Pretreated by Sodium Hydroxide
Woochul Jung, Dhanalekshmi Savithri, Ratna Sharma-Shivappa, Praveen Kolar, Sunkyu Park, North Carolina State University

10:00 am Corn fiber as a renewable resource for producing microbial lipids
Ivan Perez, Kyle Goetzelmann, Yi Cui, Yanna Liang, Southern Illinois University

10:15 am Biological Hydrogen Production from NMMO (N-Methylmorpholine-N-Oxide) Pretreated Sugarcane Bagasse
Shunchang Yang,University of Florida; Pratap Pullammanappallil,University of Florida; Robert Diltz,Air Force Research Laboratories; Subramanian Ramakrishnan,FAMU-FSU College of Engineering

10:30 am Break

10:45 am Beyond Sugar: Engineering a Carboxylic Acid Platform for Lignocellulosic Biomass
Boya Xiong, Pennsylvania State University; Tom Richard, Pennsylvania State University

11:00 am Isolation and characterization of anaerobic microorganisms from the Logan City Wastewater Lagoon System for the production of high value bioproducts
Joshua T. Ellis, Neal Hengge, Ronald C. Sims, and Charles D. Miller, Utah State University

11:15 am Anaerobic Co-Digestion of Swine Manure and Corn Stover with Additional Enzymes for Enhancing Biogas Production
Jorge Gontupil, Zhimin Liu, M. Darwin, Jay J. Cheng, North Carolina State University.

11:30 am Potential of methane production from anaerobic co-digestion of swine manure with rice straw and cocoa husk
M. Darwin, Zhimin Liu, Jorge Gontupil, Jay J. Cheng, North Carolina State University

I-D: METABOLIC PATHWAY ENGINEERING
Chair: Dr. Ryan Senger, Virginia Polytechnic Institute and State University (Virginia Tech)

9:30 am Sugar Utilization in Escherichia coli at the Single-cell Level
Taliman Afroz, Chemical and Biomolecular Engineering, North Carolina State University, Konstantinos Biliouris, Chemical Engineering and Materials Science, University of Minnesota, Yiannis Kaznessis, Department of Chemical Engineering

9:45 am Characterization of the Pradimicin A Biosynthetic Pathway
Kandy Napan, Department of Biological Engineering, Utah State University; Whitney Morgan, Department of Biological Engineering, Utah State University; Thomas Anderson, Department of Biology, Utah State University; Jon Takemoto, Department of Biology, Utah

10:00 am Host selection for synthetic pathways using a computational systems biology approach to explore biodiversity
Hadi Nazem-Bokaee, Virginia Tech; Ryan S. Senger, Virginia Tech

10:15 am Metabolic flux redistribution for enhanced production of 1, 2-propanediol and 1-propanol in Escherichia coli
Rachit Jain, University of Georgia; Yajun Yan, University of Georgia

10:30 am Break

10:45 am Deriving Metabolic Engineering Strategies with Flux Ratios Genome-Scale Modeling
Ryan S. Senger, Jiun Y. Yen, Hadi-Nazem Bokaee, Benjamin G. Freedman, and Ahmad I.M. Athamneh, Department of Biological Systems Engineering, Virginia Tech

11:00 am Pathway Pioneer: A Web-based Network Visualization and Flux Analysis Tool
Nicholas S. Flann, Utah State University; Jonathan Valiente, Utah State University; Misty Wallace, Utah State University; Richard Brown, Utah State University; Scott Hinton, Utah State University.

11:15 am Regulation of the production of antitumor chromomycins in Streptomyces roseiscleroticus
Jia Zeng, Department of Biological Engineering, Utah State University; Jixun Zhan, Department of Biological Engineering, Utah State University

11:30 am Raman spectroscopy for metabolic engineering applications
Ahmad. I. M. Athamneh and Ryan S. Senger, Department of Biological Systems Engineering, Virginia Tech

12:00 pm -1:00 pm LUNCH (on your own)

FRIDAY AFTERNOON, MARCH 8, 2013

1:10 pm – 2:40 pm GENERAL SESSION: BioBusiness Nexus
Chair: Dr. Guigen Zhang, Clemson University

Panel Theme: *Moving Technology for Translation*

Panel Members:

Terri Lomax, PhD
Vice Chancellor, Research, Innovation and Economic Development
NC State University

Jackie Quay, MSPH, JD
Interim Director of the Office of Technology Development
University of North Carolina, Chapel Hill

Bryan Baines, Rph.
Associate Director
Office of Licensing & Ventures
Duke University

Joseph Nixon, MBA
Business Development Director
North Carolina Biotechnology Center

Amanda Elam, PhD
President
Galaxy Diagnostics, Inc.

Ruth Shuman, PhD
NSF Program Director, SBIR/STTR program
Presentation: *Preparing a Winning NSF SBIR/STTR Proposal*

2:40 pm - 2:45 pm Break

2:45 pm – 4:05 pm GENERAL SESSION: Funding Opportunities for Biological Engineering
Chairs: Dr. Liju Yang, North Carolina Central University;
Dr. Eric McLamore, University of Florida

2:45 pm Funding opportunities at NSF SBIR/STTR programs
Dr. Ruth Shuman, NSF, Program Director, SBIR/STTR program

3:10 pm Introduction to NSF Chemical Measurement and Imaging Program
Dr. Lin He, NSF, Program Director, Chemical Measurement and Imaging program

3:35 pm Funding opportunities at NASA Space Life and Physical Sciences Division
Dr. D. Marshall Porterfield, NASA, Division Director, NASA Space Life and Physical Sciences, Human Exploration and Operations Mission Directorate.

4:00 pm Question & Answer Session

4:05 pm – 4:15 pm Break

4:15 pm – 6:30 pm Four Concurrent Sessions

II-A: SYNTHETIC BIOLOGY
Chair: Dr. Chase Beisel, North Carolina State University

4:15 pm Estimation of gene network parameters from single-cell fluorescence trajectories
Matthew W Lux, David A Ball, Jean Peccoud, Virginia Bioinformatics Institute

4:30 pm Development of a Biophysical Model of Translational Coupling
Tian Tian, Pennsylvania State University; Howard Salis, Pennsylvania State University

4:45 pm Engineering safeguard mechanism for microbial swarmbots
Shuqiang Huang, Duke University: Anna Jisu Lee, Duke University

5:00 pm Break

5:15 pm Synthetic Biology and Bioinformatics for Predictable Control of Therapeutic Genes
Caroline Hom, Arizona State University; Karmella Haynes, Arizona State University

5:30 pm Development of Flavin-based Fluorescent Proteins for Biological Imaging
Arnab Mukherjee, Kevin B. Weyant, Joshua Walker, Charles M. Schroeder, Department of Chemical and Biomolecular Engineering; John Ossyra, Kaustubh D. Bhalerao, Department of Agriculture and Biological Engineering, University of Illinois at Urbana-Champaign

5:45 pm Temporal control of self-organized pattern formation without morphogen gradients in engineered bacteria
Stephen Payne, Bochong Li, David Schaeffer, Lingchong You, Duke University

6:00 pm Constructing a Synthetic Gene Network to Model and Understand Signaling Interactions in Drosophila melanogaster
Ashley Jermusyk, North Carolina State University; Gregory T. Reeves, North Carolina State University

6:15 pm Economic production of Polyhydroxyalkanoates in Escherichia coli
Asif Rahman, Utah State University;Ronald C. Sims, Utah State University; Charles D. Miller, Utah State University

II-B: NANOMATERIALS AND NANOSYSTEMS
Chairs: Dr. Ming Su, University of Central Florida; Dr. Adarsh Radadia, Louisiana Tech University

4:15 pm Multiplexed detection of biomarkers using phase change nanoparticles
Chaoming Wang, Liyuan Ma, Yong Qiao, University of Central Florida

4:30 pm Top-down fabrication of particulate micro/nanodevices for drug delivery and cell tracking
Jingjiao Guan, Peipei Zhang, Junfei Xia, Zhibin Wang, Florida State University

4:45 pm Micellar nanodroplet-assisted ligand exchange of metal complex by dsDNA
Fei Yan, North Carolina Central University; Jennifer M. Romeika, North Carolina Central University

5:00 pm Break

5:15 pm Carbon Nanotubes Interfacing Bacillus anthracis Spores
Liju Yang, North Carolina Central University

5:30 pm The Effects of the Electrical Double Layer on Giant Ionic Currents through Single Walled Carbon Nanotubes
Samuel Bearden, Clemson University; Guigen Zhang, Clemson University

5:45 pm Ag-TiO2-CNT Nanoparticles for Environmental Remediation: Synthesis, Characterization and Application
Youngmi Koo, Ginaya Littlejohn, Boyce Collins, Jagannathan Sankar,Yeoheung Yun, North Carolina A&T State University; Vesselin N. Shanov and Mark Schulz,University of Cincinnati.

6:00 pm Seeded Nanodiamond Surfaces for Bacterial Biosensing
Adarsh Radadia, Louisiana Tech University

II-C: BIOENERGY: BIOCHEMICAL CONVERSIONS
Chairs: Dr. Yanna Liang, Southern Illinois University; Dr. Pratap Pullamanapalil, University of Florida

4:15 pm New insight into enzyme decrystallization of plant cellulose
Ms Xiaohui Ju, Dr. Elive Brown, Dr. Xiao Zhang, Washington State University; Dr. Mark Bowden, Environmental Molecular Science Laboratory, Pacific Northwest National Laboratory

4:30 pm The effect of water-soluble polysaccharides on enzymatic hydrolsyis of bacterial cellulose
Lin Fang, Jeffrey Catchmark, Pennsylvania State University, Department of Agricultural and Biological Engineering

4:45 pm Catalytic Oxidation of lignin to value added chemicals
Lalitendu Das, Dr. Praveen Kolar, North Carolina State University

5:00 pm BREAK

5:15 pm Development of a thermochemical process for the hydrolysis of polylactic acid for reuse
Diane Chauliac, K.T. Shanmugam, L. O. Ingram, P.C. Pullammanappallil, University of Florida

5:30 pm Influence of carbon source preadaptation on substrate utilization by Clostridium autoethanogenum
Rachel M. Slivka, Mari S. Chinn, Amy M. Grunden, North Carolina State University

II-D: ENVIRONMENTAL ENGINEERING: COMPLEXITY & SYSTEM ISSUES
Chair: Dr. Wen Zhang, University of Arkansas

4:15 pm Analysis of indirect effects within ecosystem models using pathway-based methodology
Qianqian Ma, Caner Kazanci, University of Georgia

4:30 pm Nutrient uptake and biofilm formation by Chlorella vulgaris fed with wastewater
Yogendra Kanitkar, Wen Zhang, University of Arkansas

4:45 pm Microbial biofilm proton and oxygen flux during biogenic corrosion of cement
Liqiu Cheng, Mitch House, W. Jason Weiss, Purdue University, M. Katherine Banks, Texas A&M University

5:00 pm Break

5:15 pm Antibiotic behavior in the environment and the corresponding potential to promote antibiotic resistance
Jeffrey Ullman, University of Florida; Murugan Subbiah, Texas A&M University; Douglas Call, Washington State University

5:30 pm The impact of continued nutrient enrichments on disinfection byproduct formation
Clint Mash, Thien Duc Do, Wen Zhang, Julian Fairey, University of Arkansas

5:45 pm Decentralized graywater recovery using bioreactors: effects of household-derived silver nanoparticles
Eric McLamore, M. Shupler, K. Ward, Y. Zhang, University of Florida; D. Jaroch, Purdue University

6:00 pm Assessing differences in mechanism of toxicity of ionic silver and silver nanoparticles in D. manga embryos
Matthew Stensberg, Rajtarun Madangopal, Qingshan Wei, Gowri Yale, Hugo Ochoa-Acuna, Alex Wei, Purdue University; Eric McLamore, University of Florida

6:15 pm Synthesis of formate through reduction of CO2 catalyzed by acidophilic formate dehydrogenase
Yong Hwan Kim, Department of Chemical Engineering, Kwangwoon University

6:30 pm **DINNER (on your own)**

SATURDAY MORNING, MARCH 9, 2013

7:00 am – 8:00 am **Breakfast on Own / Professional Development Forum**
Organizers: Dr. Ronald Sims, Utah State University;
Elisabeth (Libbie) Linton, WestTech Engineering, Inc, Salt Lake City, Utah

8:00 am – 9:00 am **GENERAL SESSION: Bioethics**
Chair: Dr. Praveen Kolar, North Carolina State University

8:00 am Welcome

8:05 am The Bioethics of Stem Cell Research
Kristina Dziki, University of Maryland

8:15 am Healthy Racism
Ashton Holton, Mississippi State University

8:25 am Bio-Printing: Extending Life at What Expense?
Ariel Isser, University of Maryland

8:35 am Ethics of Human Enhancement in Bioengineering
Renee Mitchell, University of Maryland

8:45 am Am I patentable? The contrasting effects of gene patents
Ryan Putman, Utah State University

8:55 am Discussion

9:00 am – 9:10 am **Break**

9:10 am – 12:00 pm **Four Concurrent Sessions**

III-A: SENSORS & BIOSENSORS 2
Chairs: Dr. Stefan Zauscher, Duke University; Dr. Amani Wan Salim, Purdue University

9:10 am Cell-based Sensing: From 2D to 3D Cell Culture
Liju Yang, North Carolina State University

9:25 am Oxygen uptake in brasicca napus (CANOLA) at or near swathing under non-lethal stress
Jeff Richards, Janelle Coutts, Levine H, Lanfang, Eric S. McLamore, University of Florida

9:40 am Lab-on-a-Chip Technology utilizing All-solid-state ion-selective electrode (ASISE) Approaches for Microfabricated Biological Sensors
W. W. Amani Wan Salim, Joon H. Park, R. Wu, M. Zeitchek, A. Brovont, A. ul Haque, S. Pekarek, M. K. Banks, Purdue University; D. Marshall Porterfield, Purdue University, NASA Headquarters

9:55 am Self-referencing Ca^{2+} sensors and differential imaging to study gravity response and physiology in Ceratopteris richardii
Masashige Taguchi, Eric McLamore, University of Florida

10:10 am Chinese Hamster Ovary Cell Culture on All-Solid-State Ion-Selective Electrodes
Joon H. Park, Dharshini Perumal, W.W. Amani Wan Salim, Zulaika Miswan, Purdue University; D. Marshall Porterfield, Purdue University, NASA Headquarters

10:25 am Break

10:40 am Paper Microfluidics Detection of Salmonella Using a Smart Phone
Tu San Park, Wenyue Li, Jeong-Yeol Yoon, University of Arizona

10:55 am Nano-Dielectrophoresis Chip Integrated with Raman Spectroscopic Self-referencing Detection of Foodborne Pathogens
Chao Wang, Chenxu Yu, Foram R. Madiyar, Jun Li, Kansas State University

11:10 am Extremely Fast Nucleic Acid Amplification by Droplet Manipulation for Point-of-Care Diagnosis of Blood Infection
Dustin K. Harshman, Roberto Reyes, Tu San Park, Jeong-Yeol Yoon, University of Arizona

11:25 am DEP Manipulation of Polystyrene Beads
Johnie Hodge, Guigen Zhang

III-B: BIOMATERIALS & STRUCTURES 2
Chair: Dr. Zhaohui Tong, University of Florida

9:10 am The impact of casein functionlized cellulose nanowhiskers on polylactic acid composites
Jin Gu, Jeffrey M. Catchmark, Pennsylvania State University

9:25 am A novel strategy for directing tissue-material interactions in surgical sealant applications
Eva Juarez Perez, Jahid Ferdous, Tarek Shazly, University of South Carolina

9:40 am Nanocrystalline cellulose for artificial vascular graft applications
Xiao Zhang, Elvie Brown, Nehal Abu Lail, Washing State University; Dehong Hu, PNNL

9:55 am Synthesis and Characterization of Nanocomposite with tunable properties of PLA-b-PDMAEMA copolymer and Caroboxymethyl Cellulose (CMC)
Nusheng Chen, Zhaohui Tong, University of Florida

10:10 am Electrical and Pneumatic Actuation of Elastomer Surfaces for Active Control of Biofouling
Phanindhar Shivapooja, Qiming Wang, Beatriz Orihuela, Daniel Rittschoff, Xuanhe Zhao, Gabriel P. Lopez, Duke University

10:25 am Break

10:40 am Computational study of lignin-protein interactions
Jyotsna L. Pandey, Heath D. Watts, James D. Kubicki, Tom L. Richard, Pennsylvania State University

10:55 am Impact of periodic injection of brilliant yellow into growing Gluconacetobacter xylinus cellulose on cellulose structure
Yuanyuan Weng, Department of Agricultural and Biological Engineering, Pennsylvania State University; Jeffrey M. Catchmark, Department of Agricultural and Biological Engineering, Pennsylvania State University

11:10 am Impact of hypoxia and physical confinement on glioblastoma cancer stem cells
Ruth Herrera-Perez, David Jaroch, Rajtarun Madangopal, Soo Ha, Kari Clase, Jenna Rickus, Purdue University

11:25 am Development of a Chemo-Mechanical Material Platform to study Neural Stem Cell Differentiation
Emily R. Geishecker, Lehigh University; Sabrina S. Jedlicka, Lehigh University

11:40 am Recognization of Poly(dimethylsiloxane) using Phage displayed Peptides
Swathi Swaminathan, Utah State University; Yue Cui, Utah State University

III-C: iGEM SYNTHETIC BIOLOGY
Chair: Dr. Tom Richard, Pennsylvania State University

9:25 am Detection of water-borne pathogens via split beta-galactosidase complementation
Khateeb Hussain, Ryan Muller, Nisarg Patel, Madeline Sands, Abhinav Markus, Ethan Ward, Rohit Rajan, Arizona State University

9:40 am Arachnicoli: Production and Purification of Spider Silk Proteins in Escherichia coli
Ryan Putman, Asif Rahman, Charles Barentine, Andrea Halling, Brian Smith, Federico Rodriguez, Elizabeth Martinez, Thomas Harris, Cameron Copeland, Cody Tramp, Joshua T. Ellis, Charles D. Miller, Utah State University; Kathleen Miller, Logan High School; Swetha Chandrasekar, Cooper Union; Jamal Abdinor, University of Utah

9:55 am Applying innovations in Human-Computer Interaction for Supporting Discovery and Learning in Synthetic Biology
Sirui Liu, Kara Lu, Linda Ding, Nicole Francisco, Veronica Lin, Casey Grote, Taili Feng, Kelsey Tempel, Michelle Ferreirae, Consuelo Valdes, Orit Shaer, Wellesley College; Nahum Seifeselassie, MIT; Heidi Wang, Stanford University

10:10 am Multiplex Automated Genome Engineering (MAGE) in Naturally Competent Bacteria
Spencer Katz, Hwa-Pyung Lim, Andriana Lebid, Jae Seong No, Aaron Lewis, Farren Isaacs, Yale University

10:25 am Break

10:40 am Real-time quantitative measurement of RNA and protein levels using fluorogen-activating biosensors
Eric Pederson , Yang Choo, Peter Wei, Jesse Salazar, Cheemeng Tan, Aaron Mitchell, Ge Yang, Catalina Achim, Diana Marculescu, Carnegie Mellon University; Natasa Miskov-Zivanov, Carnegie Mellon University, University of Pittsburgh

10:55 am Do Multiple Start Codons Affect Codon Slippage?
Hannah Jepsen-Burger, Tom Richard, Howard Salis, Pennsylvania State University

III-D: TISSUE & CELLULAR ENGINEERING
Chairs: Dr. Angela Pannier, University of Nebraska; Dr. Tarek Shazly, University of South Carolina

9:10 am Hydroxylated Flavones Reduce Alzheimer's Disease Amyloid-beta Oligomerization and Physiological Activity
Melissa A. Moss, Department of Chemical Engineering and Biomedical Engineering Program, University of South Carolina, J. Will Reed, Department of Chemical Engineering, University of South Carolina, Kayla Pate, Department of Chemical Engineering, University of South Carolina, John Clegg, Biomedical Engineering Program, University of South Carolina, Mac Rogers, Department of Chemical Engineering, University of South Carolina

9:25 am An Agent-based Model of Ductal Carcinoma in situ (DCIS) and its Validation in a Tissue-engineered Model of DCIS
Qanita BaniBaker, Soonjo Kwon, Ahmadreza Ghaffarizadeh, Gregory J.Podgorski, Nicholas S. Flann, Utah State University

9:40 am Zein: New polymer for nonviral gene delivery
Jessica D. Taylor, Mary C. Regier, Qiuran Jiang, Angela K. Pannier, University of Nebraska- Lincoln

9:55 am Effect of Media Formulation on Human Mesenchymal Stem Cells (hMSCs) Maintenance In Vitro
Meghan E. Casey, Bree Ann Young, Courtney E. LeBlon, Sabrina S. Jedlicka, Lehigh University

10:10 am Influence of alginate hydrogel biomechanical properties on the in vitro development of pre-implantation porcine embryos
Catherine N. Sargus, Angela K. Pannier, University of Nebraska-Lincoln; Elane C. Wright, Jeremy R. Miles, USDA-ARS U.S. Meat Animal Research Center

10:25 am Break

10:40 am Engineered B-Cell Biosensor for Specific, Sensitive and Rapid Detection of E. coli O157:H7
Ling Wang, Yanbin Li, University of Arkansas, Zhejiang University; Ronghui Wang, Byung-Whi Kong, Kaiming Ye, University of Arkansas

10:55 am Human Mesenchymal Stem Cell Elastic Modulus directs Differentiation Capacity
Courtney LeBlon, Caitlin Fodor, Tony Zhang, Xiaohui Zhang, Sabrina Jedlicka, Lehigh University

11:10 am Gold Nanoparticles Conjugated to Purified Collagen
Claire Spradling, Luis Jimenez, Dave Grant, Rebecca Rone, Sheila Grant, University of Missouri

11:25 am Methods for in vitro scaffold free cartilage tissue engineering
Mark L. Mosher, Steven H. Elder, Mississippi State University

11:40 am Slanted Columnar Thin Film (SCTF) Substrates for Biomolecule Delivery and Cell Culture
Tadas Kasputis, Alex Pieper, Daniel Schmidt, Derek Sekora, Keith Brian Rodenhausen, Eva Franke-Schubert, Mathias Schubert, and Angela K. Pannier, University of Nebraska-Lincoln

12:00 pm- 1:00 pm **LUNCH (on your own) / POSTER VIEWING**

SATURDAY AFTERNOON, MARCH 9, 2013

1:00 pm – 3:00 pm **GENERAL SESSION: KSBB President address/FRONTIERS IN BIOLOGICAL ENGINEERING**
Chairs: **Dr. D. Marshall Porterfield**
Purdue University/ NASA Life and Physical Sciences Division; IBE President-Elect
Dr. Liju Yang
North Carolina Central University

1:00 pm **KSBB President Address**
Dr. Seung Wook Kim, Korea University College of Engineering
Presentation: Challenges in biofuel cell: Enzymatic fuel cell

1:30 pm **(*Invited Talk*)** Fluorescent Carbon Dots for Bioimaging and Beyond
Dr. Ya-Ping Sun
Frank Henry Leslie Professor of Chemistry,
Clemson University

2:00 pm **(*Invited Talk*)** Acoustic Microfluidics and New Biofunctional Colloids for Bioanalytical Applications
Dr. Gabriel Lopez
Professor of Biomedical Engineering and Mechanical Engineering and Materials Science
Duke University

2:30 pm **(*Invited Talk*)** Engineered Biological Systems for Long Duration Human Space Exploration
Dr. D. Marshall Porterfield
Professor of Biological Engineering and Biomedical Engineering
Purdue University
Division Director, NASA Life and Physical Sciences Division

3:00 pm - 3:10 pm **Break**

3:10 pm – 4:40 pm **Two Concurrent Sessions and Poster View**

IV-A: ENVIRONMENTAL ENGINEERING: ECOLOGICAL ENVIRONMENT MODELING BIOLOGICAL ENGINEERING DESIGN
Chair: Dr. Prem Parajuli, Mississippi State University

3:10 pm Ishmael and Environmental Ethics for Biological Engineering Design
Arthur T. Johnson, University of Maryland

3:25 pm Evaluation of soil organic carbon and soil moisture content from agricultural fields
Prem B. Parajuli, S. Duffy, J. Hatten, Oregon State University

3:40 pm A Mobile Sensor for Water Quality Monitoring in Water Distribution System
Ruoxi Wu, School of Civil Engineering, Purdue University; W. W. Amani W. Salim, Departmentof Agricultural and Biological Engineering, Birck-Bindley Physiological Sensing Facility, Purdue University; Aaron Brovont, School of Electrical and Computer Engineering, Purdue University

3:55 pm An Ecophysiology Model to Link Genes to Phenotypes of the Common Bean
Melanie J. Correll, Li Zhang, Raveendra H. Patil, Kenneth J. Boote, James W. Jones, C. Eduardo Vallejos, University of Florida

4:10 pm Microbial crowd sourcing: Measuring bioavailable nutrient content in soils
Aurelein Desaunay, P. Chaturvedi, M. Taguchi, J. Ullman, Agricultural & Biological Engineering, University of Florida, J.L. Garland, Microbiological & Biological Engineering, University of Florida

4:25 pm Significance of seed culture methods on mycelial morphology and production of a novel anti-cancer anthraquinone by marine fungus Halorosellinia sp.
Chao Yu, East China University of Science and Technology

IV-B: BIOENERGY: ALGAE-BASED SYSTEMS
Chairs: Dr. Umakanta Jena, University of Georgia; Dr. Eric McLamore, University of Florida

3:10 pm Enhanced Carbon Flux for algal cultivation systems via Thin-Film Mass Transfer
Ben J. Stuart, Chalermsak Dasa-ard, David Bayless, Ohio University

3:25 pm Physiological Methods for Maximal Fatty Acid Production in Genetically Engineered Cyanobacteria
Travis Saari, Michigan State University; Victoria Work and Dr. Matt Posewitz, Colorado School of Mines

3:40 pm Nutrients recycling strategies for microalgae-based CO2 bio-mitigation system
Xinyi E, Czarena Crofcheck, University of Kentucky; Jennifer Aurandt, Kettering University

3:55 pm Extracellular organic matter (EOM) and salt effect on marine microalgae flocculation
Andrea J Garzon, Francesca Moss, and Zivko L Nikolov, Texas A&M University; Silvia Ramirez, Universidad Industrial de Santander

4:10 pm Design of a Swine Wastewater Treatment Facility to Produce Periphytic Algae as a Biomass Energy Feedstock
Nathan Holeman, M. D. Matlock, D. C. Carrier, C. V. Maxwell, W. Zhang, T. A. Costello, University of Arkansas

4:25 pm Development of an integrated algal biorefinary for polysaccharide and biofuel production
Cesar M Moreira, Yatin Behl, Murali Raghavendran, Spyros Svoronos, Edward Philips, Pratap Pullammanappallil, University of Florida

4:40 pm – 6:00 pm **Poster Competition/ General Poster Session**
Chair: Dr. Melissa Moss, University of South Carolina

NOTE: Poster presenters are permitted to set up beginning at 9:30 am on Friday, March 8, 2013. The room will be open to attendees at 12:00pm on Friday, March 8, 2013 to view the posters prior the competition at 4:40 pm on Saturday, March 9, 2013.

6:30 pm - 8:30 pm **Awards Banquet**

POSTER LISTS

General Poster Session

1. ~~**Impact of hypoxia and physical confinement on glioblastoma cancer stem cells** **(CANCELLED)**~~
   ~~Ruth Herrera-Perez (Presenting author), David Jaroch, Rajtarun Madangopal, Soo Ha, Kari Clase, Jenna Rickus, (Corresponding author), Associate Professor of Agricultural and Biological Engineering, Purdue University~~

2. **Agent-Based Models for Synthetic Biology**
   Laurie J. Heyer, A. Malcolm Campbell, Andrew Lantz, Tucker Whitesides, Jonah Galeota-Sprung, Davidson College; Todd T. Eckdahl, Jeffrey L. Poet, Missouri Western State University

3. **Coupling of single-walled carbon nanotubes with near-infrared radiation inactivates Bacillus anthracis spores and stimulates spore germinations**
   Xiuli Dong, Biomanufacturing Research Institute and Technology Enterprise (BRITE) and Department of Pharmaceutical Sciences, North Carolina Central University; Yongan Tang, Marvin Wu, and Branislav Vlahovic, Department of Physics, North Carolina Central University

4. **Development of a Cost-effective Impedance Immunosensor for Rapid and Specific Screening of Avian Influenza Virus H5N1 Asian Field Strain**
   Ronghui Wang, University of Arkansas; Xiaofei Yan, China Agricultural University; Zhanming Li, Zhejiang University; Yuntao Li, Chinese Academy of Sciences; Peirong Jiao, South China Agricultural University; Dong An, China Agricultural University; Mauhua Wang, China Agricultural University; Ming Liao, South China Agricultural University; Yanbin Li, University of Arkansas

5. ~~**X-ray excited luminescence properties and applications of Gd2O3:Eu nanophoshors**~~
   ~~Chaoming Wang, Ming Su, University of Central Florida~~ **(MOVED TO GRADUATE COMPETITION)**

6. **Mesophilic Anaerobic Co-Digestion of Swine Manure with Swithgrass and Wheat Straw for Methane Production**
   Zhimin Liu, Jorge Gontupil, Mr. Darwin, Angelica Pura,Jay J.Cheng, North Carolina State University

Undergraduate Student Poster Competition Session

1. **Physiological Methods for Maximal Fatty Acid Production in Genetically Engineered Cyanobacteria**
   Travis Saari, Michigan State University; Victoria Work, Colorado School of Mines; Dr. Matt Posewtiz, Colorado School of Mines

2. **Baseline evaluation of groundwater quality in central New York in the face of shale gas development**
   Anne Elise Creamer, Lauren McPhillips, Cornell University Department of Biological and Environmental Engineering; M. Todd Walter, Cornell University Department of Biological and Environmental Engineering, Cornell University Soil and Water Lab

3. **Using E. coli to Determine Optimal DNA Design for Metabolite Production**
   Ben Clarkson, Becca Evans, Betsy Gammon, Meredith Nakano, Caroline Vrana, Laurie J. Heyer, A. Malcolm Campbell, Davidson College
   Caleb Carr, David Carr, Eddie Miles, Jerrad Morton, Sachith Polpitaya, Kamay Trueblood, Todd T. Eckdahl, Jeffrey L. Poet, Missouri Western State University

4. **Sustainable Green Roof Irrigation using Wastewater**
   Samuel Frey, Environmental Engineering Department, University of Connecticut; J. Suen, R. Munoz-Carpena, Agricultural and Biological Engineering Department, University of Florida; E.S. McLamore, Agricultural and Biological Engineering Department, University of Florida

5. **Developing Assembly Methods for Genetic Circuits used to Optimize Metabolic Pathways**
   Ben Clarkson, Becca Evans, Betsy Gammon, Meredith Nakano, Caroline Vrana, Laurie J. Heyer, A. Malcolm Campbell, Davidson College
   Caleb Carr, David Carr, Eddie Miles, Jerrad Morton, Sachith Polpitaya, Kamay Trueblood, Todd T. Eckdahl, Jeffrey L. Poet, Missouri Western State University

6. **A Nano-Zeolite Sensor to Detect Surfactants, a Contribution to Microbial Remediation Feasibility Studies**
   Katelyn S. Ward, Dr. Eric S. McLamore, Prachee Chaturvedi, Stephanie Burrs, Shige Taguchi, Diana Vegas, University of Florida

7. **A Nanomaterial-Mediated Biosensor for Measuring Sarcosine**
   Grace Justinvil, Stephanie L. Burrs, Diana Vanegas, Eric S. McLamore, University of Florida

8. **Effectiveness of Phenolic Acids Derived from Coconut Oil on Amyloid-beta Inhibition**
   Steven Vance, University of Kentucky/University of South Carolina

9. **Bioenergy Landscape Design to Minimize Cultivation Emissions and Production Expenses**
   Thai N. Dinh, University of Oklahoma; John L. Field, Colorado State University; Keith H. Paustian, Colorado State University

10. **Arachnicoli: Production and Purification of Spider Silk Proteins in Escherichia coli**
    Ryan Putman, Asif Rahman, Charles Barentine, Andrea Halling, Brian Smith, Federico Rodriguez, Elizabeth Martinez, Thomas Harris, Cameron Copeland, Cody Tramp, Joshua T. Ellis, Charles D. Miller, Utah State University; Kathleen Miller, Logan High School; Swetha Chandrasekar, Cooper Union; Jamal Abdinor, University of Utah

Graduate Student Poster Competition Session

1. **Simulation of micro particle movement and alignment in an electric field**
   Yu Zhao, Johnie Hodge, Guigen Zhang, Clemson University, Bioengineering department, Clemson University

2. **The Effect of Agricultural-Based Nitrogen Sources on Production of Biohydrogen by Thermotoga Neapolitana**
   Louis Hill, Caye Drapcho, Clemson University

3. **Development of Flavin-based Fluorescent Proteins for Biological Imaging**
   Arnab Mukherjee, Kevin B. Weyant, Joshua Walker, John Ossyra, Kaustubh D. Bhalerao, Charles M. Schroeder (corresponding), Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign

4. **Cellular Responses to Anti-cancer Drug in 3D and 2D Cell Cultures**
   Goral Trivedi, William Tyson, Liju Yang, North Carolina Central University

5. **Effect of Gold/Copper Sulfide Core/Shell Nanoparticles on Bacillus Anthracis Spores**
   Addae Ebenezer, Marquita Lilly, Eric McCoy, North Carolina Central University; Chang Yang, Wei Chen, Physics, University of Texas at Arlington

6. **Pinewood activated char for mitigation of p-cresol**
   Lalitendu Das, Dr. Praveen Kolar, Dr. John. J. Classen, Dr. Jason A. Osborne, North Carolina State University

7. ~~**Optrode biosensors for in vivo sucrose monitoring in plants** **(CANCELLED)**~~
   ~~Leyla Nesrin Kahyaoglu, Rajtarun Madangopal, Cliff Weil, Jenna L. Rickus, Purdue University~~

8. ~~**Electroactive Polymer-based Nanocomposites For Multi-analyte Amperometric Biosensors** **(CANCELLED)**~~
   ~~Rajtarun Madangopal, Matthew C. Stensberg, Nicholas Pulliam, D. Marshall Porterfield, Jenna L. Rickus, Purdue University~~

9. ~~**Nature-inspired porous silica biomaterials for precision size exclusion at the mammalian cell surface** **(CANCELLED)**~~
   ~~Jennifer L. Kahn, Jenna L. Rickus, Purdue University~~

10. **Oxygen consumption as a rapid bioindicator of changes in water quality using Daphnia magna embryos**
    Matthew Stensberg (Presenting), Michael Zeitchek, Kul Inn, Maria Sepulveda, D. Marshall Porterfield (Corresponding), Purdue University; Eric McLamore, University of Florida at Gainesville

11. **Solvent Selection and Recovery for Liquid-Liquid Extraction of Acetic Acid and Water**
    Mahdieh Aghazadeh, Abigail Engelberth, Purdue University

12. **Engineered B-Cell Biosensor for Specific, Sensitive and Rapid Detection of E. coli O157:H7**
    Ling Wang, Yanbin Li, University of Arkansas, Zhejiang University; Byung-Whi Kong, Ronghui Wang, Kaiming Ye, Sha Jin, University of Arkansas

13. **Nanobead and aptamer based QCM biosensor for rapid detection of avian influenza virus**
    Luke Brockman, Ronghui Wang, Jacob Lum, Lisa Kelso, and Yanbin Li, University of Arkansas

14. **Investigation of Media Ingredients and Water Sources for Algae CO2 Capture at Different Scales to Demonstrate the Correlations Between Lab-scale and L**
    Tabitha Graham, Czarena Crofcheck, Aubrey Shea, Michael Montross, University of Kentucky Biosystems and Agricultural Engineering; Mark Crocker, University of Kentucky Center for Applied Energy Research, Rodney Andrews, University of Kentucky Center for Applied Energy Research, Biosystems and Agricultural Engineering

15. **Evaluation of the Antimicrobial Properties and Biocompatibility of Polypropylene Mesh Conjugated with Gold Nanoparticles**
    Ross Hartter, Dr. Sheila Grant, Dr. Shramik Sengupta, University of Missouri

16. **Economic production of Polyhydroxyalkanoates in Escherichia coli**
    Asif Rahman, Ronald C. Sims, Charles D. Miller, Utah State University

17. **Characterization of the herboxidiene biosynthetic gene cluster in Streptomyces chromofuscus ATCC 49982**
    Jia Zeng, Lei Shao, Jiachen Zi, Jixun Zhan, Department of Biological Engineering, Utah State University

18. **Characterization of the Pradimicin A Biosynthetic Pathway**
    Kandy Napan, Whitney Morgan, Jixun Zhan, Department of Biological Engineering, Utah State University; Thomas Anderson, Jon Takemoto, Department of Biology, Utah State University

19. **Isolation and characterization of anaerobic microorganisms from the Logan City Wastewater Lagoon System for the production of high value bioproducts.**
    Joshua T. Ellis, Neal Hengge, Ronald C. Sims, and Charles D. Miller, Utah State University

20. **Phycocyanin Production by Cyanobacterial Biofilms Cultured in Oilfield Wastewater (Produced Water)**
    Jonathan Wood, Ronald Sims, Jon Takemoto, Dong Chen, Utah State University

21. **Antisense RNA: A Metabolic Switch for Controlling the Gene Expression**
    Hadi Nazem-Bokaee, Ryan S. Senger, Virginia Tech

22. **Fine-tuning Bacterial Gene Expression using Antisense RNA**
    Hadi Nazem-Bokaee, Ryan S. Senger, Virginia Tech

23. **Thermoresponsive Pervaporation Membranes Enabled by Hyperbranched Polyglycerols and Elastin Like Protein Conjugates**
    Juliet Kallon and Darlene Taylor, North Carolina Central University

24. **X-ray excited luminescence properties and applications of Gd2O3: Eu nanophosphors**
    Chaoming Wang, Ming Su, University of Central Florida

INVITED SPEAKERS

Keynote Speaker
Randolph V. Lewis, Ph.D.
Utah State University

Dr. Randolph V. Lewis received his bachelor's degree from CalTech in 1972 and his M.S.in 1974 and PhD in 1978 degrees from the University of California at San Diego. He was a postdoctoral fellow at the Roche Institute of Molecular Biology. He joined the faculty at U. of Wyoming in 1980 and was Professor of Molecular Biology until 2011. He served as Department chair for five years and as a special assistant to the Vice President for Research. Randy joined Utah State University in 2011 as USTAR Professor of Biology and in the Synthetic Biomanufacturing Center.
His group has published over 130 papers in a wide variety of journals and has written 16 book chapters. They have seven issued patents. He has had grants totaling over $31 million. Dr. Lewis has had 18 PhD and 3 M.S. students and currently has six PhD students and 16 undergraduates.

His research focuses on spider silks and the proteins they are made from. In the past 20 years they identified, through DNA cloning, the proteins that make up all six of the different silks which spiders can make as well as the glue proteins. With that information they proposed the key parts of the proteins that are responsible for the high elasticity and the tensile strength of these fibers. Based on that data they constructed synthetic genes that make proteins in which the elastic or strength elements have been systematically varied. These proteins have been produced in bacteria, purified and are now being spun in to fibers to determine the effects of the different elements. They currently can produce fibers that have energies to break greater than Kevlar and steel. The goal of this work is to provide a method to produce fibers with custom designed strength and elasticity for applications such as ligament and tendon repair/replacement, high tech clothing, parachutes, etc. Their research has been featured on several TV shows including Discovery, Nova, BBC and CSI New York.

Erik Reimhult, Ph.D.
University of Natural Resources and Life Sciences, Vienna, Austria

Professor Erik Reimhult is head of the Laboratory for Biologically inspired Materials (BIMat) and the Department of Nanobiotechnology (DNBT) at the University of Natural Resources and Life Sciences Vienna (BOKU), Austria. He got his PhD in Physics and Engineering Physics in 2004 from Chalmers University of Technology, Sweden, on self-assembly of supported lipid assemblies and biosensor development. He has worked as postdoctoral researcher at the Institute of Materials Research and Engineering Singapore, and as senior scientist (Oberassistent) at the Department of Materials at the ETH Zurich on biointerfaces, biosensing, nanofabrication, polymer surface modifications, supramolecular assembly, and multi-functional nanoparticles. In 2010 he became full professor at the BOKU and in 2012 Prof. Reimhult was recently awarded an ERC Starting Grant Award for research on NP-lipid membrane interactions. The current focus of research of the BIMat is the synthesis and properties of core-shell NPs, membrane

interactions, colloid strings and self-assembled and magnetically actuated membranes, investigated with colloid and surface sensitive techniques.

Suzie H. Pun, Ph.D.
University of Washington

Dr. Suzie H. Pun received her Chemical Engineering Ph.D. degree in 2000 from the California Institute of Technology. She then worked as a senior scientist at Insert Therapeutics for 3 years before joining the Department of Bioengineering at University of Washington (UW). She is currently the Robert J Rushmer Associate Professor of Bioengineering, an Adjunct Associate Professor of Chemical Engineering, and a member of the Molecular Engineering and Sciences Institute at UW. Her research focus area is in drug and gene delivery systems and she has published over 50 research articles in this area. For this work, she was recognized with a Presidential Early Career Award for Scientists and Engineers in 2006.

Ruth Shuman, Ph.D.
National Science Foundation

Dr. Ruth Shuman joined the National Science Foundation in August 2009. She is currently serving as Program Director for the Biology and Chemical Technologies (BC) Cluster in the SBIR/STTR Program, and was named Cluster Leader in 2011. Her area of technical focus at NSF is biological and biomedical technologies, and she has a keen interest in synthetic biology and metabolic engineering. Formerly, she was the founder, president, and CEO of a successful venture-backed life science company, Gentra Systems, Inc., that developed, manufactured, and sold products for genetic testing and research to clinical and research laboratories worldwide. Following Gentra's acquisition by Qiagen, she held various consulting/advisory positions with start-up companies, and was CEO-In-Residence for Life Science with the University of Minnesota's Venture Center evaluating the business potential of University-developed technology. Ruth began her career as a faculty member at North Carolina State University, and was a pioneer in the development of gene transfer and genetic engineering technology. She holds a Ph.D. from the University of Minnesota in the area of Genetics and Cell Biology.

Lin He, Ph.D.
National Science Foundation/ North Carolina State University

Prof. Lin He is currently holding an Associate Professorship at the Department of Chemistry at the North Carolina State University and an Adjunct Professorship at Department of Biomedical Engineering, a joint program between UNC/NC State University. She is also a rotating Program Director in the Chemistry Division at the National Science Foundation.

Prof. He received her B.S degree from Peking University in China and her PhD from Penn State University in Analytical Chemistry. After graduation, she worked for Surromed, Inc, a biotech startup company in the bay area, before she joined NC State in 2003. Prof. He's research interests include development of new biosensing tools using radical polymerization and exploitation of Ordered Nanoarray-Assisted Laser Desorption/ Ionization Mass Spectrometry in metabolite profiling and chemical imaging. During the past year and half, Prof. He has been managing research portfolios within the Chemical Measurement and Imaging (CMI) program and the Macromolecular, Supramolecular, and Nanochemistry (MSN) program in the CHE division at NSF.

D. Marshall Porterfield, Ph.D.
NASA headquarters/Purdue University

Dr. D. Marshall Porterfield is Division Director for Space Life and Physical Sciences at NASA headquarters in Washington DC where he oversees the Human Research, Physical Sciences, and Space Biology Programs. The division includes the designee NASA liaison for the International Space Station National Lab, management of extramural grants and research, as well as the intramural research and engineering assets at six NASA centers. Currently the programmatic focus is on ISS utilization. At Purdue University Dr. Porterfield is a Professor of Biological Engineering where he helped found the Physiological Sensing Facility at Discovery Park. His expertise is development of sensing technologies as tools for research in biology, agriculture, the environment, space, and medicine using scanning probe sensors, biosensors, bio-MEMS, bio-nanotechnology, biomimetics and lab-on-a-chip technologies. His work in gravitational and space biology includes cell signaling and biophysical phenomena. He has received numerous awards including the Halstead Young Investigator Award from the American Society for Gravitational and Space Biology and election to the College of Fellows for the American Institute for Medical and Biological Engineering for his work. His leadership includes service as President of the American Society for Gravitational and Space Biology, and recently was elected to serve as President for the IBE.

Seung Wook Kim, Ph.D.
Korea University, Korea

Dr. Seung Wook Kim is the president of Korean Society for Biotechnology and Bioengineering(KSBB) and the Professor in the Department of Chemical and Biological Engineering at Korea University. He earned his B. S. in Chemical Engineering from Korea University, Seoul, Korea, in 1980; and also his M. S. in Chemical Engineering from Korea University in 1984; and his Ph. D. in Chemical Engineering at the University of Birmingham, Birmingham, U. K. in 1989. Before joining the faculty at Korea University in 1996, he worked at the Department of Genetic Engineering, the University of Suwon. Dr. Kim's primary line of research is based on bioprocess engineering; bioreactor design and bioprocess optimization involving bioenergy production, biocatalysis, protein and DNA immobilization on nanomaterials, enzymatic biofuel cell, microchannel bioreactor, bioreactions with supercritical fluid, rheological study of various fungi, and strain development by genetic modification. Dr. Kim has over 110 publications in international refereed journals, and over 30 patents.

Ya-Ping Sun, Ph.D.
Clemson University

Prof. Ya-Ping Sun earned his Ph.D. at the Florida State University in 1989. After postdoctoral training at the University of Texas at Austin, he joined the Clemson faculty as an assistant professor in 1992 and was promoted to full professor in 1999. Since 2003, he has been the endowed Frank Henry Leslie Chair Professor of Natural and Physical Sciences. His research interest is in the development of nanomaterials and other novel materials for various technological applications. Dr. Sun has more than 280 publications in journals and books.

Gabriel P. López, Ph.D.
Duke University

Prof. Gabriel P. López is founding Director of the NSF's Research Triangle Materials Research Science and Engineering Center (MRSEC) and a Professor of Biomedical Engineering and Mechanical Engineering & Materials Science at Duke University. He is also Research Professor of Chemical Engineering and a member of the Center for Biomedical Engineering at the University of New Mexico. In 1991, he completed Ph.D. studies in chemical engineering at the University of Washington where he worked under the mentorship of Prof. Buddy D. Ratner as a Kaiser Aluminum Co. Graduate Fellow. From 1991-1993, he was an NIH and Ford Foundation Postdoctoral Fellow under the mentorship of Prof. George M. Whitesides in the Dept. of Chemistry at Harvard University. He was appointed Assistant Professor of Chemical Engineering and Chemistry at the University of New Mexico in 1993, promoted to Associate Professor in 1999, and promoted to the rank of Professor in 2004. He was the founding director (2005) of the UNM Center for Biomedical Engineering. His research is currently supported by several sources including the NSF, NIH, DOD, and DOE. His current research interests include biointerfacial phenomena,

bioinspired and biomimetic materials and bioanalytical microsystems to address problems in medicine, biotechnology and environmental quality.

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TRIANGLE MRSEC | Materials Research Science and Engineering Center

The Triangle Materials Research Science and Engineering Center (MRSEC), launched in September 2011, is a national resource for materials science and engineering research and education located in the Raleigh/Durham/Chapel Hill area of North Carolina. The MRSEC research team encompasses faculty and students at Duke University, North Carolina State University, North Carolina Central University and the University of North Carolina-Chapel Hill. The MRSEC will have a major national and international impact in soft matter materials science through generation of new fundamental insights and theoretical understanding, new design principles, and new applications and uses for colloidal and macromolecular materials and their higher order assemblies

MEETING PROGRAM

Technical Sessions:

General Sessions

- BioBusiness Nexus: *Moving Technology for Translation*
- Funding Opportunities for Biological Engineering
- Frontiers in Biological Engineering

Concurrent Sessions (Oral)

- Bioenergy: Algae-Based Systems
- Bioenergy: Biochemical Conversions
- Biomaterials & Structures
- Environmental Engineering: Ecological Environment Modeling; Biological Engineering Design
- Environmental Engineering: Complexity & System Issues
- iGEM Synthetic Biology
- Metabolic Pathway Engineering
- Nanomaterials and Nanosystems
- Sensors & Biosensors
- Synthetic Biology
- Tissue & Cellular Engineering

Poster Session

- General Poster Presentations
- Student Poster Competition

Welcome from the IBE President and Program Co-Chairs

Welcome to the 2013 Annual Conference of the Institute of Biological Engineering. This year's meeting in Raleigh, North Carolina will be the 18th annual conference of our dynamic and vibrant community of biological engineers. We come together to share our exciting advances in research, innovations in education, success stories in economic development, to learn from our colleagues and reconnect with friends. In doing so, we come together as an institution to shape the profession of biological engineering.

For our keynote presentation, we are privileged to host Dr. Randolph V. Lewis, of Utah State University. His presentation, titled *Spider Silk: Developing an Ancient Biomaterial for the Future,* will take place on Friday morning, March 8th.

We also extend a warm welcome to the leadership of the Korean Society of Biotechnology and Bioengineering (KSBB) and affirm a continuation of scholarly exchanges between IBE and KSBB. Dr. Seung Wook Kim of Korea University, President of the KSBB will present an introduction to KSBB on Saturday, March 9th.

We look forward to several more dynamic general sessions. On Friday, Dr. Ruth Shuman (NSF SBIR/STTR), Dr. Lin He (NSF Chemical Measurement and Imaging Program), and Dr. D. Marshall Porterfield (NASA Space Life and Physical Sciences Division), IBE President-Elect, will be featured. These speakers will cover *Funding Opportunities for Biological Engineering* at NSF and NASA.

Dr. Porterfield will join Dr. Yaping Sun and Dr. Gabriel Lopez as part of our *Frontiers of Biological Engineering* Series on Saturday. This session will cover topics that are at the forefront of biological engineering and represents the next generation of research topics.

IBE greatly values and celebrates the efforts and successes of our student members. We will recognize regional meeting winners, finalists in the 2013 Bioethics Essay Competition, winners in the undergraduate and graduate Student Poster Competitions, student travel awardees, iGEM participants and student presenters.

We express our sincere gratitude to all the presenters and speakers for bringing their research and sharing their excitement about biological engineering at this meeting. We encourage and invite you to submit your original research presented here for publication in the *Journal of Biological Engineering*. A special "thank you" to session chairs, judges, program committee, council members and the headquarters staff (especially Brian Doty and Courtney Devine) for their effort and initiative to make this meeting happen.

We look forward to celebrating with you, from the reception on Thursday through the Awards Banquet on Saturday. Thank you for making IBE a success!

Czarena Crofcheck,
President

Eric McLamore,
Program Co-Chair

Liju Yang,
Program Co-Chair

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Challenges in biofuel cell: Enzymatic fuel cell

Invited Talk: KSBB President Address

Hee Uk Lee, Seung Wook Kim

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Enzymatic fuel cells (EFCs) use redox enzymes with high electron transfer rates that lead to high power density from bioavailable substrates. However, an EFC has a short lifetime and poor power density, both of which are related to enzyme stability, electron transfer rate, and enzyme loading. Recent progress in nanobiocatalysis opens the possibility to improve in these aspects.

In this study, a bioelectrode technique that employs mediater, which uses electron transferring agents, was developed for an EFC. The EFC was also improved for efficient wiring of the enzymes. Efficiency of the EFC was evaluated under mild conditions, such as ambient temperature, neutral pH, and component concentrations. The electrical properties of a basic EFC based on a redox enzyme system as biocatalysts were investigated. These results are expected to find wide applications such as biosensor, Lab-on-a-chip, and detection system. This straightforward EFC process could lead to other fuel cells.

IBE Abstract # 559

Fluorescent Carbon Dots for Bioimaging and Beyond

Frontiers in Biological Engineering

Ya-Ping Sun, Clemson University

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Abstract

Semiconductor quantum dots (QDs), especially the highly fluorescent CdSe-based core-shell nanostructures, have generated much excitement for their variety of potential applications in optical bioimaging and beyond. These QDs are widely considered as being more advantageous over conventional organic dyes as well as genetically engineered fluorescent proteins in terms of optical brightness and photostability. However, a serious disadvantage with these popular QDs is their containing heavy metals such as cadmium, whose significant toxicity and environmental hazard are well-documented. Alternative benign (nontoxic) QD-like fluorescent nanomaterials have therefore been pursued, including our reported finding of fluorescent carbon nanoparticles (dubbed "carbon dots"). Carbon dots are surface-passivated small carbon nanoparticles, where the surface passivation is most effective via functionalization with organic or bio-molecules. In addition to sharing some of the major advantageous characteristics of semiconductor QDs, including high photostability, large two-photon excitation cross-sections, and applicability as optical imaging agents in vitro and in vivo, carbon dots are also non-blinking, readily water-soluble, and nontoxic according to currently available cytotoxicity and in vivo (mice) toxicity results. In this talk the current status on the development and understanding of carbon dots will be presented and discussed.

IBE Abstract # 423

Physiological Methods for Maximal Fatty Acid Production in Genetically Engineered Cyanobacteria.

Bioenergy: Algae-Based Systems

Travis Saari, Michigan State University; Victoria Work, Colorado School of Mines; Dr. Matt Posewitz, Colorado School of Mines, Michigan State University

Corresponding Author: Travis Saari, Undergraduate Researcher, Michigan State University, 140 Collingwood Dr., East Lansing, Michigan 48823

Abstract

Photosynthetic microorganisms such as cyanobacteria and green-algae are an attractive prospect for the production of sustainable bioproducts and biofuels for many reasons, including metabolic efficiency and genetic amenability. Strains of the cyanobacterium Synechococcus sp. PCC 7002 have been genetically modified to produce and excrete medium-chain fatty acids suitable for use as fuel precursors. It has been shown that in green algae, physiological conditions such as nutrient limitation can cause significant changes in metabolism and carbon storage (Work et al., 2011). The fatty-acid secreting cyanobacteria mutants had not been studied in this way. In an effort to both understand the mutant metabolism and increase their FFA production, the effects of various physiological conditions on cyanobacterial growth and free fatty acid (FFA) production were assessed. Nutrient limitation had only neutral or negative effects on growth and productivity, unlike previous findings in algal systems. In contrast, both high light intensity and urea utilization treatments showed increased FFA production, which did not directly correspond to cell density. Studies in Synechococcus have linked lipid peroxidation to high intensity light and, more unexpectedly, urease activity (Maeda et al., 2008) (Sakamoto, 1998). Thus, the trends in FFA production may be related to the effects of lipid peroxidation on the mutants, and they support the proposed mechanism of the mutants FFA-secretion involving impaired membrane lipid-recycling.

IBE Abstract # 517

DEVELOPMENT OF AN INTEGRATED ALGAL BIOREFINARY FOR POLYSACCHARIDE AND BIOFUEL PRODUCTION

Bioenergy: Algae-Based Systems

Cesar M Moreira, University of Florida (Presenting), Yatin Behl, University of Florida; Murali Raghavendran; University of Florida; Spyros Svoronos; University of Florida, Edward Phlips, University of Florida; Pratap Pullammanappallil, University of Florida

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Abstract

The use of algae as an alternative source of bioenergy (e.g., biogas or bioethanol) has a large potential. Viewing algae as a photosynthetic unit for production of biomass, it could potentially provide billions of tons of biomass without compromising food supply or agricultural land. Microalgae are microscopic plants that are primary synthesizers of organic matter in aquatic environment. They have high surface to volume ratio enabling the rapid uptake of nutrients and CO2, and, have a faster cell growth rate than land based plants. However, algae biomass for energy is still in its infancy. Key issues affecting large scale algae based production of bioenergy are: selection of species, cultivation and harvesting techniques and conversion technology. This research investigated the cultivation and harvesting of unique hypersaline nitrogen-fixing exopolysaccharide producing cyanobacteria Synechococcus sp and methods to increase the exopolysaccharide production by light exposure and intensity, and, CO2 concentration. We obtained a maximum growth rate of 0.48 days-1 using air and a 13/11 hour light/dark cycle. Polysaccharide production increased when using high concentrations of CO2 in headspace and 24 hours light exposure during stationary growth phase.

IBE Abstract # 523

Extracellular organic matter (EOM) and salt effect on marine microalgae flocculation

Bioenergy: Algae-Based Systems

Andrea J Garzon, Texas A&M University; Silvia Ramirez, Universidad Industrial de Santander; Francesca Moss, Texas A&M University, and Zivko L Nikolov, Texas A&M University, Biological and Agricultural Engineering

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Abstract

Harvesting and dewatering of algal biomass is one of the costly steps in the recovery of algal oil. Several different technologies, including inorganic electrolytes and positively charged polymers, have been successfully applied for harvesting of fresh-water microalgae by flocculation; however, their effectiveness with marine microalgae are believed to be limited by the salt content present in the growth media. This work is meant to understand the impact of extracellular organic matter vs. salt concentration during flocculation of two marine microalgae strains: Nannochloris oculata and Nannochloropsis salina. Aluminum chloride (AlCl3) and five cationic polymers of different molecular weight and charge density level were used as flocculation agents and the effect of flocculant dosage, salt concentration, and media pH were analyzed. In general, polymer flocculation of marine microalgae was more efficient when using high charge density polymers (99% charged), and the dosage was affected by the amount of extracellular organic matter present in the culture. Extracellular organic matter increased the flocculant dosage requirement about 5-fold when compared to algal cultures under the same conditions without EOMs. To determine the effect of salt concentration when no EOMs were present, two different salt concentrations (5 and 35 g/L NaCl) were tested at a flocculant dosage of 4 mg/L. There was no significant difference in biomass removal efficiency indicating that extracellular organic matter was the main cause for higher flocculant dosage requirements. Acidification of algal cultures with HCl before flocculation with inorganic salt (AlCl3) was also compared; Nannochloropsis salina cultures required 21-fold less acid to reach the optimal flocculation pH than Nannochloris oculata cultures. Polymer flocculation did not required pH adjustment, with the exemption of chitosan, which performed better at pH 8.0.

IBE Abstract # 527

Nutrients recycling strategies for microalgae-based CO2 bio-mitigation system

Bioenergy: Algae-Based Systems

Xinyi E, University of Kentucky; Czarena Crofcheck, University of Kentucky; Jennifer Aurandt, Kettering University

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Abstract

Microalgae are potential microorganisms for CO2 bio-mitigation. Scenedesmus was cultivated in air-lift tubular closed bioreactors, designed to be fed on flue gases from coal-fired power plant. Due to the fast growing nature of microalgae as well as the scale of operation, substantial amounts of nutrients and water are required, producing a significant amount of biomass. Anaerobic digestion is a practical approach to decompose microalgae biomass and produce methane and other mineralized nutrients (e.g. N and P). The correlation between organic loading rate and biogas production rate was studied in a continuously-stirred anaerobic digester. To improve the degradability, the biomass of Scenedesmus was thermal-chemically pretreated prior to anaerobic digestion. Four factors of pretreatment were investigated: algae harvesting (gravity settlement, flocculated algae, flocculated and dried algae), temperature (50 or 90°C), alkaline dosage (0, 3, 6 or 12% of algae DW), and pretreatment duration (10, 30 or 60 min). Overall pretreatment rendered approximately 6~16% of cell materials soluble in water, which indicated the breakdown in cell wall structure. The treated microalgae slurry was supplied to the microalgae cultivation media, and showed potential as a media supplement for Scenedesmus growth.

IBE Abstract # 539

Design of a Swine Wastewater Treatment Facility to Produce Periphytic Algae as a Biomass Energy Feedstock

Bioenergy: Algae-Based Systems

Nathan Holeman, University of Arkansas; M. D. Matlock, University of Arkansas; D. C. Carrier, University of Arkansas; C. V. Maxwell, University of Arkansas; W. Zhang, University of Arkansas; T. A. Costello, University of Arkansas

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Abstract

Water bodies in northwest Arkansas are enriched with nitrogen and phosphorus due to land use activities in urban and agricultural areas. Animal manure management systems often use land application to fertilize pastures and row crops, with nutrient residues moving to surface waters during storm events. Despite this direct nutrient transport pathway, current agricultural waste management practices can be adapted to reduce environmental contamination and better utilize the nutrients and energy embodied in animal manures. Attached (or periphytic) algae growth can be used to treat swine wastewater to utilize excess nitrogen and phosphorus and to produce a renewable bioenergy feedstock. With the partial removal of nitrogen and phosphorus due to algal uptake, agricultural waste holding ponds could be better managed with potential for less negative environmental impact. With the concurrent production of a biomass feedstock, the whole farm system could claim bio-energy production which offsets fossil fuel use and results in a net reduction in greenhouse gas production. To test practical large scale implementation of this methodology, a wastewater treatment facility that is intended to produce attached algae was constructed at the University of Arkansas Swine Finisher Unit, near Savoy, Arkansas. The system consists of a 61 m X 7.6 m (200 ft X 25 ft) flow-way, multiple pumps to aid in water circulation, an automated pump control system, and a mechanized algae harvest sub-system. The system has four parallel flow paths (for testing multiple wastewater sources simultaneously) along a precision grade of 2%. The circulation flow capacity is 379 L/min (100 gpm) per flow path. Algae will grow and attach to commercial indoor-outdoor carpet used as growing medium. Algae grown in this facility will be characterized and processed for use as a biofuel feedstock. This research will provide data on water and

energy balances, productivity, capital and operating costs, so that a life cycle assessment of the system sustainability, from environmental and economic viewpoints, can be performed. Although the system is hydraulically operational, algal production has not yet been characterized. This paper will focus on system design, construction, operation and control of the hydraulic components. The system design is scalable to larger areas and would be applicable to the processing of natural surface waters in addition to wastewater inflows to produce biomass feedstock.

IBE Abstract # 561

Enhanced Carbon Flux for Algal Cultivation Systems via Thin-Film Mass Transfer

Bioenergy: Algae-Based Systems

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Abstract

The delivery of aqueous carbon to algal cultivation systems can be enhanced through air sparging, or even more so by sparging with CO2 enriched gas (air or flue). Issues related to carbon introduction through sparging include the proximity of a high CO2 emitting source, cost of compression and/or transport (if the source is not co-located with the growth system), capture efficiency in the aqueous phase, maximizing the interphase mass transfer area, the potential for fouling and system maintenance requirements, potential for introduction of other gas constituents (contaminants) to the media, and the creation of low pH microenvironments with the potential to inhibit growth or that require significant chemical addition for sufficient buffering capacity. As reported at the 2012 IBE Annual Meeting, a nutrient delivery header and supported fabric membrane have been employed to supply dissolved carbon to both open (raceway) and closed (tubular photobioreactor) algae cultivation systems. Total inorganic carbon (TIC) measurements demonstrated mass transfer rates and saturation concentrations that exceed those predicted by conventional thin-film mass transfer and thermodynamic models, and have resulted in growth rate improvements over comparable systems not enriched with CO2. Using the flow controlling header, carbon is introduced into the growth system at or near the saturation concentration of the film volume, but at a dilution rate that does not negatively impact bulk media pH. This paper will present the final mass transfer data and impacts on algal growth rates as a function of media composition (RO water vs. defined media vs. artificial sea water), temperature, partial pressure of CO2 delivered, pH, buffer concentration (NaOH), and available membrane surface area.

IBE Abstract # 453

Potential of methane production from anaerobic co-digestion of swine manure with rice straw and cocoa husk

Bioenergy: Biochemical Conversions

M. Darwin, North Carolina State University; Zhimin Liu, North Carolina State University; Jorge Gontupil, North Carolina State University; Jay J. Cheng, North Carolina State University

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Abstract

The performance of an anaerobic digestion process depends on the composition of the material to be digested. This work investigates the methane production potential of the anaerobic co-digestion of swine manure with rice straw and cocoa husk, and key factors related to temperature and solid content responsible for enhancing co-digestion performance are examined. To evaluate the stability of anaerobic co-digestion process of swine manure with rice straw and cocoa husk, batch reactors utilized were operated in triplicate. The process was carried out in 500 ml batch reactors under constant temperature and agitation. Temperature was set under mesophilic condition at 35 °C; and agitation speed was set at 300 rpm. Culture utilized as inoculums for the batch experiment was taken from semi-continuously operated reactors, with 25 days of hydraulic retention time under mesophilic conditions. Based on the results from co-digestion of rice straw with swine manure using 2 percent of total solids, it shows that there was an increase of more than 90 % in the methane production compared with a digester fed only with swine manure. Furthermore, culture added with rice straw generated average methane production at about 1,421 mL, after 38 days of digestion. On the other hand, swine manure used as a control produced 706.5 mL of methane on average after 38 days. It occurred as swine manure has high nitrogen content. However, rice straw has low nitrogen content, and high carbon content. Therefore, by adding rice straw in the culture, it may enhance buffering capacity in the anaerobic digestion. Keywords: Methane production, anaerobic co-digestion, rice straw, cocoa husk, swine manure

IBE Abstract # 460

Anaerobic Co-Digestion of Swine Manure and Corn Stover with Additional Enzymes for Enhancing Biogas Production

Bioenergy: Biochemical Conversions

Jorge Gontupil, North Carolina State University; Zhimin Liu, North Carolina State University ; M. Darwin, North Carolina State University; Jay J. Cheng, North Carolina State University

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Abstract

Anaerobic co-digestion of swine manure and corn stover using NS81210 cellulase complex and NS81220 protease for enhancing biogas production has been studied in two completely-mixed and semi-continuously fed reactors (Reactor 1 and Reactor 2). Each reactor had a working volume of 14 liters and was operated at 35°C, a hydraulic retention time of 25 days, and an agitation speed of 200 rpm. Both reactors were fed with swine manure and corn stover. Every day 560 ml of swine wastewater and 14 g of corn stover were added to Reactor 1 and Reactor 2. Temperature was set at 35°C and stirrer at 120 rpm. Biogas generation and pH in both reactors were measured every day. After the performance of both reactors reached steady state, 140 mg of cellulase (1% of TS) was added to reactor 2 to determine the effect of this enzyme in biogas production. Average biogas production was 5627 ml in Reactor 1, and 6355 ml in Reactor 2 which produced 11% more biogas when cellulase complex was added. Gas analysis showed a methane content of 44.2% in Reactor 1 and 43.2% in reactor 2, which gives an average methane production of 2487 ml and 2745 ml in Reactor 1 and Reactor 2 respectively (9% more methane production when cellulase was used). Currently, the same conditions are being applied and the same procedure is being followed to study the effect of protease in biogas production. An increase on biogas generation is expected when protease is added to the reactor.

IBE Abstract # 500

Beyond Sugar: Engineering a Carboxylic Acid Platform for Lignocellulosic Biomass

Bioenergy: Biochemical Conversions

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Abstract

Acidogenic digestion is a variant of anaerobic digestion, truncated before methanogenesis to produce carboxylic acids as the primary product. These short chain organic acids can serve as reactive intermediates for downstream lignocellulosic biofuel and biochemical production. As a mixed-culture, non-sterile process, acidogenic digestion may provide a low-cost strategy for high substrate conversion rates and high mass yield coefficients from lignocellulosic biomass. This study investigated several process parameters, including solid loading rate, inoculum loading rate, retention time, and nutritional requirements during batch fermentation of pretreated switchgrass. Inoculum originally derived from rumen fluid, silage and compost was recycled and blended with pretreated switchgrass at inoculum ratios of 1:90, 1:30, and 1:10, with total solids loading ranging from 2.5 g l-1 to 250 g l-1 (all values on a dry basis). Optimum conditions were found at a solids loading of of 10 g l-1. Bacterial growth and community were investigated by molecular techniques including 16s rRNA sequencing and real time PCR to gain more knowledge about the acidogen behavior in response to pH and solid loading rate in the undefined and complex system. These studies will also offer the information of dominant bacterial populations for future inocula improvement. By coupling assessment of microbial community dynamics with conventional bioprocess development, we hope to use biological engineering analysis to accelerate the development of a robust and scalable process.

IBE Abstract # 513

Isolation and characterization of anaerobic microorganisms from the Logan City Wastewater Lagoon System for the production of high value bioproducts

Bioenergy: Biochemical Conversions

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Abstract

The ability to engineer novel systems for the production of high value products such as biosolvents, bioacids, and biogas from cheese whey, a waste feedstock rich in lactose and protein, has been demonstrated. Several clostridia species were isolated from the anaerobic sediments of the Logan City Wastewater Lagoon System. Isolates were obtained by counter-selecting against non-spore forming bacteria, diluted to extinction, and streaked for isolation several times to ensure purity. Isolation was confirmed by amplifying the 16S rRNA subunit and comparing the sequences to the NCBI database. Phylogenetic analysis of these isolates indicates that they are closely related taxonomically to Clostridium butyricum, Clostridium metallolevans, and Clostridium bifermentans, while numerous others were not phylogenetically similar to characterized organisms within the NCBI database indicating that these isolates may indeed be novel. The production of high value bioproducts using cheese whey has been quantified using GC and HPLC. Initial studies have shown hydrogen production yields to be 1.2 mol-H2/mol-lactose, while ethanol production was 2.1g/L, with a volumetric ethanol productivity of 0.058 g/Lh, as well as the production of lactate, butyrate, and acetate from certain isolates. Our goal is to demonstrate the feasibility of understanding relationships within a complex anaerobic community to aid in isolating microbes with physiologies of interest to engineer novel strategies for the production of high value and renewable bioproducts.

IBE Abstract # 525

Corn fiber as a renewable resource for producing microbial lipids

Bioenergy: Biochemical Conversions

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Abstract

Corn fiber is a mixture of corn hulls and residual starch not extracted during the milling process. Depending on the corn variety, corn fiber makes up 8-11% of the dry weight of corn kernel. Corn fiber consisting of approximately 70% of carbohydrates, namely cellulose, starch and hemicelluose can serve as a renewable resource for biofuel production. Producing bioethanol from corn fiber has been evaluated in the past years. As US hits the ethanol blend wall, other biofuels rather than ethanol needs to be produced. This talk will focus on converting corn fiber to microbial lipids. Similar to generating biofuels from any lignocellulosic feedstocks through the biochemical pathway, a series of steps, such as pretreatment, hydrolysis and fermentation is needed. This presentation will cover our recent work on corn fiber to lipids including pretreatment by lime, fermentation by an oleaginous yeast, Cryptococcus curvatus, and lipid extraction using microwave irradiation.

IBE Abstract # 529

Correlation between Lignin Monomers Recovery and Fermentable Sugars Generation from Miscanthus Pretreated by Sodium Hydroxide

Bioenergy: Biochemical Conversions

Woochul Jung, Biological and Agricultural Engineering, North Carolina State University; Dhanalekshmi Savithri, North Carolina State University; Ratna Sharma-Shivappa, North Carolina State University; Praveen Kolar, North Carolina State University; Sunkyu Park, North Carolina State University

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Abstract

Lignin is the most recalcitrant aromatic polymer that impedes release of fermentable sugars from lignocellulosic biomass. The main lignin aromatic monomers of interest are syringyl and guaiacyl propanol. Yet, the effect of changes in lignin monomer content (during pretreatment) on subsequent sugar production is little known. Sodium hydroxide pretreatment of Miscanthus giganteus at different alkali concentrations (0.5%, 1.0% and 1.5%) and pretreatment times (15 min, 30 min and 60 min) at 121 oC /15 psi followed by enzymatic hydrolysis was investigated to analyze fermentable sugars generation (glucose, xylose and arabinose). Nitrobenzene oxidation (NBO) and high performance liquid chromatography (HPLC) are being used to measure changes in lignin monomers between raw and pretreated miscanthus for relative syringaldehyde/vanillin (S/V) ratio. Concurrent studies on lignin monomer content in switchgrass indicate that S/V ratio of samples treated at 0.5% NaOH at 15 min was higher than that of raw biomass by 38%. The correlation between lignin monomer content and fermentable sugars released via hydrolysis will be investigated to clarify the fundamental bioconversion mechanism of the pretreatment. Basic understanding of changes in lignin monomer distribution needed to develop transgenic varieties that are more suitable to bioconversion will also be gained.

IBE Abstract # 554

Biological Hydrogen Production from NMMO (N-Methylmorpholine-N-Oxide) Pretreated Sugarcane Bagasse

Bioenergy: Biochemical Conversions

Shunchang Yang, University of Florida; Pratap Pullammanappallil,University of Florida; Robert Diltz,Air Force Research Laboratories; Subramanian Ramakrishnan,FAMU-FSU College of Engineering, University of Florida

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Abstract

Concerns about global warming, climate change and energy security have brought about a renewed interest in renewable energy. Considerable attention has been devoted towards renewable energy from biomass. Among all the biofuels, hydrogen is the most attractive since it combusts cleanly producing only water and has high energy content. There are various technologies for producing hydrogen like electrolysis, thermolysis, photocatalytic and fermentation processes. There is considerable interest in hydrogen production by dark (in the absence of light) fermentation process due to its higher efficiency and smaller footprint compared to other biological routes. A mixed anaerobic bacterial culture is used as an inoculum for this fermentation. The culture is subjected to cycles of heat treatment (120°C for 2 hrs) and fermentation to select for hydrogen producing Clostridia and inactivate hydrogen consuming syntrophic bacteria and methanogens. Soluble feedstocks have been successfully used as a substrate for hydrogen fermentation. Lignocellulosic biomass is the most abundant bioresource on the world. The inaccessibility of the lignin matrix and the crystalline structure of cellulose are obstacles for microbial breakdown of this substrate. The feedstock requires pretreatment to saccharify the cellulosic components prior to fermentation. This study, investigated the effect of pretreatment using 86% N-methylmorpholine-N-oxide (NMMO) as solvent at ambient pressure and temperature of 130°C for enhancing the bio-hydrogen potential of sugarcane bagasse. NMMO pretreatment was compared with dilute acid pretreatment. In this paper we will present the characteristics of biomass after pretreatment, yield of hydrogen, composition of hydrogen in biogas, soluble byproducts of hydrogen fermentation and feasibility of converting the fermentation by products subsequently to methane.

IBE Abstract # 566

Advances in the analysis of sweet sorghum composition for bioprocess development

Bioenergy: Biochemical Conversions

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Abstract

Sweet sorghum is being developed as a source of sugars for the production of biofuels and other products by fermentation processes. The plant produces large quantities of small saccharides that are directly accessible by fermentation microbes, as well as polysaccharides, primarily cellulose, which may be converted into accessible form. The analytical methods commonly applied to determine the composition of sorghum are, as with other types of biomass, ultimately derived from the Klason sulfuric acid hydrolysis method, as recently compiled by NREL. Though the basic elements of the procedure may be applied to any type of biomass, practically speaking, the characteristics of the biomass being studied may dictate some modification. Sweet sorghum offers a particular set of challenges for these methods. The high sugar and moisture content of the pith and the impermeability of the rind cause post-harvest sample degradation and difficulty in achieving sufficient drying. The high soluble sugar content increases the variability in the moisture content of the dried material and can interfere with the results of the lignocellulose hydrolysis if not fully extracted. The lignocellulosic composition of the stalk varies radially, and this variation is tied strongly to variation in the density of the dried tissue. In addition, grinding the dried stalk material results in a wide range of particle sizes. The density and particle size variability can cause difficulty in obtaining representative subsamples, and in ensuring consistency during extraction and acid hydrolysis. We have developed a set of procedures to account for the particular nature of sweet sorghum, and generate accurate composition data for use in the development of this promising feedstock.

IBE Abstract # 534

Ishmael and Environmental Ethics for Biological Engineering Design

Biological Engineering Design

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Abstract

The possible spectrum of biological systems as the objects of biological engineering designs is very broad, and may include some very sensitive environmental issues. Most ethical discussions are very broad and vague, not providing clear answers to questions about choices related to the best design for the circumstances. *Ishmael* is an influential 1992 novel by Daniel Quinn about a Socratic discussion between a man, as a student, and a gorilla, as the teacher who can communicate telepathically. The book poses the questions: "With man gone, will there be hope for gorilla [as a surrogate for the natural world]?" and "With gorilla gone, will there be hope for man?" It's an interesting book that posits that once man developed agriculture he upset the natural balance and started the world on a path toward environmental disaster. Although the book is structured as a series of questions and answers between the teacher and student, it can lead to a strategy for ethical choices related to biological engineering designs that have environmental impact. That is, whatever the choice, make as little negative impact as possible on the world as it would naturally exist. It's an interesting view, and argues for minimal domination and control by humans over nature, especially those design solutions that benefit one species (humans) to the detriment of all others.

IBE Abstract # 557

Significance of seed culture methods on mycelial morphology and production of a novel anti-cancer anthraquinone by marine fungus Halorosellinia sp.

Biological Engineering Design

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Abstract

The effects of seed culture methods on the mycelial morphology and production of a novel promising anti-cancer anthraquinone 1403C by marine mangrove saprophytic fungus Halorosellinia sp. (No. 1403) was investigated. Inoculums were prepared using different seed culture methods, i.e., mycelia obtained by grinding biomass that was harvested from baffled flask culture (M1); biomass harvested from baffled flask culture (M2); biomass obtained from unbaffled flask culture with glass beads (M3); biomass attained from unbaffled flask culture (Control). The corresponding fermentations using M1, M2 and M3 enhanced 1403C production by 243.5%, 194.8% and 70.2%, respectively, as compared to that using Control (0.33±0.03 g/L). Interestingly, 1403C production increased with the increase of ratio of number of clumps to pellets. Maximum 1403C production from baffled flask cultures was 4.8-fold of that from unbaffled flask culture. Increasing shaking speed from 170 rpm to 260 rpm could highly improve 1403C production by 151.8%.

IBE Abstract # 415

Peptide-based Materials For Drug Delivery

Biomaterials and Structures

Suzie Pun, David Chu, Maryelise Cieslewicz, Anthony Convertine, Philip Horner, Don Maris, Joan Schellinger, Drew Sellers, Julie Shi, Patrick Stayton, Hua Wei, University of Washington

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Abstract

Peptides are versatile materials that can possess biological function and also be easily synthesized and incorporated into biomaterials. In this talk, I will summarize our work in bioactive peptide identification, synthesis of peptide-based polymers, and applications of these materials for drug delivery. Peptides for cell-specific targeting and endosomal release have been identified by phage library selection and by evaluating virus-derived peptides, respectively. For drug and gene delivery applications, these peptides incorporated into well-defined peptide-based polymers using RAFT polymerization. We have synthesized polymers that can be degraded by disulfide bond reduction and also by enzymatic cleavage. The polymers were tested in vitro to neuron-like, differentiated PC-12 cells and by direct brain injection. We demonstrate that peptide-based copolymers are versatile materials that can be tuned for efficient gene transfer with low accompanying toxicity.

IBE Abstract # 426

The impact of casein functionlized cellulose nanowhiskers on polylactic acid composites

Biomaterials and Structures

Jin Gu, Pennsylvania State University; Jeffrey M. Catchmark, Pennsylvania State University

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Abstract

Cellulose fibers have been widely used as reinforcements for green polymer composites. The dispersion of hydrophilic cellulose fibers in the hydrophobic polymer matrix is usually poor. In this study, the biodegradable composites of poly(lactic acid)(PLA) incorporating cellulose nanowhiskers (CNWs) with the goal of improving filler dispersion and mechanical performance have been investigated. Whole milk casein protein was used as a dispersant due to its unique features. Casein proteins contain both hydrophobic and hydrophilic domains and could bind to both cellulose and polylactic acid. Using casein as a dispersant could maintain the environmental benefit of the composites as well. Casein functionalized CNWs (CS-CNW) were mixed with PLA in dimethylformamide in order to get a well dispersed suspension. PLA composites were prepared with 2%, 5% and 10% CS-CNW or CNW filler loading. The CNWs were fluorescence stained and their dispersion in the PLA matrix was studied under a fluorescence microscope. The dispersion of CS-CNWs in PLA was better than the control. Tensile tests of all the samples were performed and the mechanical performance of the PLA-CS-CNW was improved at certain conditions.

IBE Abstract # 427

MicroSCALE screening reveals genetic modifiers of therapeutic response in melanoma

Biomaterials and Structures

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Abstract

Cell microarrays are a promising tool for performing large-scale functional genomic screening in mammalian cells at reasonable cost. Due to technical limitations, however, their use has been restricted to a narrow range of cell lines and short-term assays. Here, I describe MicroSCALE (Microarrays of Spatially Confined Adhesive Lentiviral Features), a cell microarray-based platform that enables application of this technology to a wide range of cell types and longer term assays. MicroSCALE was used to uncover kinases that when overexpressed partially desensitized B-RAFV600E-mutant melanoma cells to inhibitors of RAF, MEK1/2, mTOR, and PI3-kinase. These screens suggested that cells treated with inhibitors acting through common mechanisms were affected by a similar profile of overexpressed proteins. In contrast, screens involving inhibitors acting through distinct mechanisms yielded unique profiles, a finding that has potential relevance for small molecule target identification and combination drugging studies. Further, by integrating large-scale functional screening results with cancer cell line gene expression and pharmacological sensitivity data, the NF-ºB pathway was validated as a potential mediator of resistance to MAPK pathway inhibitors. The MicroSCALE platform described here may enable new classes of large-scale, resource-efficient screens that were not previously feasible, including those involving combinations of cell lines, perturbations, and assay outputs or those involving limited numbers of cells and limited or expensive reagents.

IBE Abstract # 434

A novel strategy for directing tissue-material interactions in surgical sealant applications

Biomaterials and Structures

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Abstract

The deployment of adhesive sealants can significantly augment available wound repair techniques and improve soft tissue healing following surgical interventions. However, soft tissue surface chemistry and mechanical loading conditions significantly vary among potential applications and confound the development of a universal adhesive material with acceptable clinical performance. Current sealants are not designed on a tissue-specific basis and are generally limited by a tradeoff between high adhesion strength and biocompatibility. We hypothesize that rational tuning of bioreactive chemical group distribution along constituent polymer chains can facilitate optimal tissue-material interactions without inducing a concomitant reduction in material biocompatibility. To enable the assessment of the proposed material design strategy, a series of two-component, aldehyde-mediated adhesive materials are synthesized using dextran aldehyde and chitosan polymers. In this experimental material system, both cohesive cross-linking within the material and adhesive cross-linking to local tissue surfaces is achieved through free aldehyde-mediated imine bond formation. Within the material series, the distribution of aldehyde groups along the constituent polymer chains are varied while keeping the total aldehyde concentration at a constant level. We quantified material properties with relevance to sealant applications, including gelation time, elastic modulus, adhesion strength and interfacial morphology, with the latter two considered following material application to renal artery, heart, kidney and lung tissue surfaces. Results show that material bioreactive group distribution strongly modulates adhesive interactions in a tissue-specific manner. Optimizing material bioreactive group distribution in a tissue-specific manner could provide a means to circumvent the persistent tradeoff between adhesion and biocompatibility.

IBE Abstract # 436

Substrate Topography Influences the Functional Neurons Produced by Direct Reprogramming of Fibroblasts

Biomaterials and Structures

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Abstract

Cellular reprogramming holds tremendous potential for cell therapy and regenerative medicine, especially true for combating neurodegenerative diseases. Recently fibroblasts have been converted into induced neurons by overexpressing the transcription factors *Ascl1*, *Brn2* and *Myt1L*. *In vivo* the microenvironment regulates cellular function ranging from adhesion and migration to proliferation. It presents physical and biochemical cues that influence cellular behavior. Here we investigate how substrate topography influences reprogramming of fibroblasts to induced neurons. Polystyrene substrates imprinted with microgratings or circular posts were used with non-patterned polystyrene samples as controls. The number of neurites per soma on the 5 mm gratings topography was significantly reduced with 1.14 neurites per soma compared to the smooth control. The corresponding average length of the neurites was 296.6 mm versus 174 mm. When comparing mRNA expression of iNs on smooth substrates to 5 □m gratings we detected 81 differentially expressed genes, including ones implicated in neuronal differentiation and cell projection organization like artemin, netrin, slit3, Thy1, growth arrest specific protein 1, and sphingosine-1-phosphate receptor 1. As characterized by electrophysiology, the average membrane potential of iNs on smooth substrates was similar to iNs on 5 □m substrates; with an average resting membrane potential of -76 mV for iNs on smooth and -82 mV for iNs on 5 mm gratings. This study advocates a role of cell-topography interactions in shaping iN identity. It also suggests the potential of applying topographical cues to optimize the microenvironment for various transdifferentiation processes.

IBE Abstract # 449

Computational study of lignin-protein interactions

Biomaterials and Structures

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Abstract

Lignin is a natural cell wall polymer found in land plants and is a major component of lignocellulosic biomass, which has long been the most prevalent biomaterial in human use, and is currently being developed as a potential biofuel and biochemical resource. The structure of lignin is very poorly understood, limiting the development of effective methods to use it in advanced composites and to convert lignocellulosic biomass to sugars and other biochemicals. Several lignin modification approaches aim at introducing peptides within the plant cell wall that strongly and specifically bind to lignin. Once introduced into the lignin matrix, such peptides could be tagged with visualization markers to aid in scientific understanding, or be targeted with proteases to make cell wall modification easier. The goal of this research is to develop efficient methods to study lignin-protein interactions using density functional theory and ultimately to use these methods to design short peptide linkers that interact strongly with lignin in plant cell walls. Lignin is primarily comprised of the 4-hydroxyphenylpropanoid monomers coniferyl alcohol (MG), sinapyl alcohol (MS) and p-coumaryl alcohol (MH). The Î²-O-4 dimer is the predominant linkage found in lignin; therefore, this dimer was used as a lignin proxy to evaluate potential intermolecular interactions between lignin and peptide proxies. Phenylalanine, tryptophan, and tyrosine are aromatic amino acids that could have potentially strong intermolecular interactions with the aromatic moieties of lignin; therefore, these amino acids are the peptide proxies for this work. Strongly interacting peptides can be used to functionalize AFM tips or localize other tags for studying cell wall structure and formation, or as linkages to control lignin polymerization and depolymerization. Our studies show that computational calculations can play an important role in understanding and characterizing lignin-protein interactions that take place inside the plant cell wall.

IBE Abstract # 450

Exploiting Softlithographic Techniques to Make Particles for Applications in Life and Material Sciences

Biomaterials and Structures

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Abstract

Nanotechnology has the potential to provide critical improvements and innovation in both life science and material science research. Progress in nanotechnology has been limited by challenges associated with controlling particle size, shape, deformability, chemical composition, uniformity, cell targeting and the ability to tune and control the release of known amounts of cargo. Particle Replication in Non-Wetting Templates (PRINT®) fabricates 'calibration quality' particles to assess the role of individual components in nanoparticle characteristics and behavior. PRINT is a continuous, roll-to-roll, high resolution molding technique that allows the fabrication of precisely defined nanoparticles with complete control over key particle attributes. The master templates are made using state-of-the-art technologies from the semiconductor industry that enables highly uniform populations of shape controlled particles to be fabricated. The top-down particle fabrication technique of PRINT enables the simultaneous and independent control over particle size (20 nm to >100 micron), particle shape (filaments, cubes, cylinders, discs, toroidal), particle composition (organic/inorganic, solid/porous), particle cargo (hydrophilic or hydrophobic therapeutics, biologics, oligonucleotides, siRNA, imaging agents, sensors, imaging agents), particle modulus (stiff, deformable), and particle surface chemistries (targeting peptides, antibodies, aptamers, cation/anion exchange, stealth PEG chains). Nanoparticle drug delivery systems can preferentially deliver and release a therapeutic cargo in the optimal dosage range, at the desired site of delivery to improve efficacy and reduce toxicity. Poly(lactic acid-co-glycolic acid) (PLGA) is highly amenable to the PRINT process and has enabled the fabrication of particles with many different chemotherapy drugs. PLGA PRINT particles containing 5 to 40 % (w/w) of docetaxel display higher *in*

vitro efficacy than the clinical formulation of docetaxel, Taxotere. PLGA particles encapsulating and coated with the cationic lipid DOTAP, showed knockdown of therapeutically relevant genes following internalization in numerous cancer cell lines. Hydrogel particles containing a reductively labile disulfide bond have been used to selectively release siRNA when delivered intracellularly. In order to create a series of particles with tunable release characteristics, acid labile silyl ether crosslinkers with precise degradation profiles have been used in hydrogel particles. Increasing the steric bulk of the substituents on the silicon atom, increased degradation rates. The ability to reduce drug toxicity through a nanoparticle formulation and predict the fate of particles as a function of physical parameters will allow for the tuning of the pharmacokinetic profiles of nanoparticle based therapeutics to target and treat specific cancers. The PRINT soft lithography technique can also be used to fabricate surfaces with anti-biofouling characteristics. Dimethacryloxy-functionalized perfluoropolyether crosslinked (PFPE) with PEG oligomers were able to inhibit the attachment of algal cells and cypris larvae of barnacles to PRINT patterned surfaces versus PFPE control surfaces. The control over the PRINT particle composition gives insight into the self-assembly processes and provides new methods for drug delivery, photonics, electronics, magnetic colloids, surface coatings and nanomachines.

IBE Abstract # 457

Multifunctional thermo-responsive polymer brushes with dynamic nanotopology for attachment, killing and release of bacteria

Biomaterials and Structures

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Abstract

In this report, we describe engineered surfaces with switchable functionality based on nanopatterned thermo-responsive poly (N-isopropylacrylamide) (PNIPAAm) brushes, which were fabricated via interferometric lithography (IL) combined with surface-initiated polymerization. The thermo-responsive conformational changes of PNIPAAm brushes were characterized by atomic force microscopy in water at different temperatures and significant topographical differences were observed for samples prepared under different IL exposure dose. For one such sample, at 25°C (below the lower critical solution temperature (LCST) of PNIPAAm), the surface was relatively smooth due to lateral crowding of PNIPAAm brushes. However, when the temperature increased to 37°C (above the LCST), a pattern appeared. This temperature triggered expanded/collapsed conformational change provides the ability to spatially regulate concealment and exposure of molecules that are immobilized on the intervals between patterned brushes. We choose a biocidal agent, quaternary ammonium salt silane (QAS), to demonstrate the utility of nanopatterned PNIPAAm brushes to control the interfacial interaction with bacteria. QAS was integrated into polymer-free regions of the substrate between nanopatterned PNIPAAm brushes and E. coli was used as a model bacteria. We found that above the LCST, collapsed and hydrophobic PNIPAAm chains facilitate the attachment of bacteria and expose QAS moieties to kill adhered bacteria, while below the LCST, swollen and hydrophilic PNIPAAm chains promote release of dead bacteria. Our results demonstrate that this nanopatterned PNIPAAm/QAS hybrid surface exhibits controllable attachment, killing and release ability in response to temperature.

IBE Abstract # 491

Impact of hypoxia and physical confinement on glioblastoma cancer stem cells

Biomaterials and Structures

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Abstract

Glioblastoma multiforme is the most aggressive type of brain cancer in humans. Despite chemical treatments and surgical removal, glioblastoma shows high recurrence and very low survival rate. Recent evidence has pointed out the importance of a small fraction of cells termed cancer stem cells (CSC). These cells have the ability to recreate the tumor and play an important role on sustaining tumor growth. CSC development is also suggested to be deeply influenced by the environment, especially by the presence of a hypoxic niche. However, most of the CSC studies have been done using two-dimensional culture, therefore ignoring the importance of physical constraints and oxygen restrictions of the real tumor environment. In this study we use silica sol-gel encapsulation to analyze the effects of physical confinement and oxygen diffusion limitations on the survival ability and metabolic protein expression of human glioblastoma CSC population (CD133+). Cell viability and mitochondrial activity are tested by CellTracker Green CMFDA and MitoTracker (Invitrogen) respectively; and global proteomic profile of CSC encapsulated cells is compared to the proteomic profile of two-dimensional cultured CSC cells. We predict a change in the proteomic expression and tumorigenic potential of CSC related to oxygen availability and the physical location of CSCs in the encapsulated neurosphere. Also, we expect dramatic changes in the survival ability of the cells in the innermost core, as this resembles the morphology of the necrotic core of GBM tumors in vivo.

IBE Abstract # 499

Electrical and Pneumatic Actuation of Elastomer Surfaces for Active Control of Biofouling

Biomaterials and Structures

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Abstract

Formation of biofilms by micro- and macro- fouling organisms is ubiquitous in nature and often referred to as biofouling. Biofouling is of critical concern due to its deleterious effects, especially in medical devices and marine environments. While the use of conventional heavy-metal based coatings have been restricted due to negative health and environmental impacts, existing environmental antifouling technologies generally rely on static surfaces, which have limited overall effective performance in field studies. Inspired by certain biological surfaces which resist microbial adhesion through active deformation and motion, we report that dynamic change of surface area and topology of elastomers can be used as a general, environment-friendly approach for effectively detaching fouling organisms. In this work, we employed electrical and pneumatic actuation of elastomer surfaces and coatings that proved to show efficient release (> 90 %) of tenaciously adhered biofouling layers and organisms. The results successfully validate our proposed hypothesis that the deformation of the elastomer surface, and not the presence of the electric field, causes biofilm detachment. Also, we demonstrate for the first time, the antifouling capabilities of dynamic surfaces actuated by pneumatic networks in laboratory and natural-marine environments. The proposed bio-inspired, dynamic surfaces can be fabricated over large areas through simple and practical processes. This new mechanism can be used in complementary with existing materials and other methods for effective control of biofouling.

IBE Abstract # 503

Impact of periodic injection of brilliant yellow into growing Gluconacetobacter xylinus cellulose on cellulose structure

Biomaterials and Structures

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Abstract

It has been demonstrated cellulose produced by Gluconacetobacter xylinum in the presence of cellulose binding dyes shows a decreased crystallinity and disrupted bundle formation. Among varieties of cellulose binding dyes, brilliant yellow, which is a kind of direct cellulose dye with molecular weight of 624.55 shows a higher ability to decrease the crystallinity of bacterial celllose. Brilliant Yellow also was found to be much more effective at reducing crystallinity than calcoflour. In this study, bacterial cellulose was produced by Gluconacetobacter xylinum (ATCC 53582) in the presence of brilliant yellow under static condition. X-ray diffraction analysis shows that the crystallinity of BC decreases considerably with increasing concentration of brilliant yellow. In addition, preliminary data suggests that the introduction of brilliant yellow also impacts crystallization preferentially in the cellulose 100 plane, perhaps due to a preferred binding of brilliant yellow to the 100 plane. Also, with a novel culture system which can periodically inject the concentrated brilliant yellow solution to the growing culture, we are trying to understand can we force the bacterial cellulose to form periodically disordered structure and study changes in the structural characteristics of cellulose under periodic injection of brilliant yellow. If amorphous cellulose is formed due to the accumulation of strain in the cellulose crystal, the introduction of periodically spaced highly amorphous regions may initially increase crystallinity. In addition, the minimum injection period coupled to the rate of polymerization may provide information on the cellulose precrystallization length.

IBE Abstract # 518

Nanocrystalline cellulose for artificial vascular graft applications

Biomaterials and Structures

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Abstract

The potential of applying nanocrystalline cellulose (NCC), an emerging renewable bio-nanomaterial, with fibrin matrix to synthesize a nanocomposite for small-diameter replacement vascular graft (SDRVG) application was demonstrated. Periodate oxidation of NCC can augment reactive carbonyl groups on NCC and facilitate the cross linking between NCC and fibrin. NCC/fibrin nanocomposite was synthesized with fibrin as the matrix and oxidized NCC (ONCC) homogeneously dispersed in the matrix. The maximum strength and elongation of the nanocomposites were determined from Atomic Force Microscopy (AFM) to and compared with a native blood vessel. The manipulation of degree of oxidation of NCC and the NCC/fibrin weight ratio resulted in the variation of strength and elongation of the nanocomposites, indicating that the nanocomposites can be tailored to conform to the diverse mechanical properties of native blood vessels. A mechanistic understanding of ONCC and fibrin interaction at molecular level was illustrated. This study established a fundamental base to utilizing nanocrystalline cellulose for practical SDRVG applications.

IBE Abstract # 535

Development of a Chemo-Mechanical Material Platform to study Neural Stem Cell Differentiation

Biomaterials and Structures

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Abstract

Cellular processes such as adhesion, proliferation, and differentiation are controlled in part by cell interactions with the microenvironment. Cells can sense and respond to a variety of stimuli, including soluble and insoluble factors (such as proteins and small molecules) and externally applied mechanical stresses. Mechanical properties of the environment, such as substrate stiffness, have also been suggested to play an important role in cell processes. The roles of both biochemical and mechanical signaling in fate modification of stem cells have been explored independently. However, very few studies have been performed to study well-controlled chemo-mechanotransduction. The objective of this work is to design, synthesize, and characterize a chemo-mechanical substrate capable of encouraging neuronal differentiation of two cell lines, C17.2s and P19s. Polyacrylamide (PA) gels of varying stiffnesses are functionalized with mixed adhesive peptides, derived from fibronectin and laminin. Peptides containing binding sequences from fibronectin (AYAVTGRGDSPASA), laminin (ADPGYIGSRGAA), and EGF repeats from laminin and tenascin (ANDNIDPNAVAA) were synthesized using 9-fluorenylmethyloxycarbonyl (Fmoc) solid state synthesis. These peptides were conjugated to the poly(acrylamide) surfaces using Sulfo-SANPAH, an amine-reactive and light-activatable crosslinker. The matrix elasticity and peptide concentration were individually modulated to systematically probe the effects of chemo-mechanical signaling on differentiation of C17.2 and P19 cells. The amount of peptide bound to the PA was determined using X-ray photoelectron spectroscopy. Nanotopographical features of the PA were examined using atomic force microscopy and gel stiffness was confirmed using a combination of rheological and microindentation techniques. Cell adherence was assayed using phase contrast microscopy and cell area measurements. In this study, a platform to study the combination of biochemical and mechanical signaling in fate modification of neural stem cells was developed and

characterized. Future studies will employ this material platform to further study the combinatorial roles of surface chemistry and elastic modulus in NSC differentiation.

IBE Abstract # 552

Synthesis and Characterization of Nanocomposite with Tunable Properties of PLA-b-PDMAEMA Copolymer and Carboxymethyl Cellulose (CMC)

Biomaterials and Structures

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Abstract

CMC based nanocomposites have been mainly limited by its hydrophilicity, leading to incompatible with hydrophobic biopolymers, such as PLA (poly lactic acid). In this project, the quaternized and hydrophilic PDMAEMA (poly 2-dimethyl-amino ethyl methacrylate) is proposed to be compatibilizer between hydrophilic CMC and hydrophobic PLA to overcome the nanoscale aggregation and the hydrophilicity of CMC. Meanwhile, our concept also includes the use of soft domain (PDMAEMA micelle structure) as the binder between stiff microfibril and PLA. The PLA composite properties can be tuned by changing CMC ratio, the molecular weight of PLA and PDMAEMA. Different molecular weight of PLA-b-PDMAEMA (poly lactic acid block poly 2-dimethyl-amino ethyl methacrylate) copolymers was prepared through ring opening polymerization and following ATRP (atom transfer radical polymerization) reaction. The successful synthesis of PLA-b-PDMAEMA with different molecular weight was confirmed by 1H-NMR (Nuclear Magnetic Resonance) and Size Exclusion Chromatograph (SEC). Then, quaternization was conducted to obtain the cationic PLA-b-PDMAEMA copolymer, which can disperse in the water to form a micelle structure. Its hydrodynamic radius was determined by Dynamic light scattering (DLS). After that, different molar ratio of cationic PLA-b-PDMAEMA and anionic CMC were combined together through ionic interaction to form biomimetic nanocomposite. The physical properties and morphologies of PLA nanocomposite film were measured by dynamic mechanical analysis (DMA) and Scanning Electronic Microscopy (SEM). Its water absorbance was measured by COBB test.

IBE Abstract # 555

Nano-engineered Polymer Coatings and Capsules for Controlled Drug Delivery and Cell Transplantation

Biomaterials and Structures

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Abstract

Macromolecular self-assembly and bio-inspired fabrication of novel biologically-active and stimuli-responsive nanomaterials are of interest for various advanced applications in bio- and nanotechnology. This talk will focus on functional ultrathin polymeric coatings and microcontainers (capsules) obtained by hydrogen-bonded layer-by-layer assembly of water-soluble polymers and biological macromolecules on solid templates, inorganic particles, and living cells. We will present pH-sensitive multilayer films of silk fibroin assembled with synthetic macromolecules and a natural polyphenol; and demonstrate that cubical, spherical, and platelet capsules with silk-containing walls can be constructed through film assembly on particulate sacrificial templates. We will also introduce routes for designing hydrogel capsules with stimuli-triggered shape transitions. Cubical hydrogel capsules with ultrathin hydrogel walls are obtained as hollow replicas of cubical sacrificial templates by chemical cross-linking of precursor hydrogen-bonded multilayers. The dynamic shape and size responses of hydrogel capsules triggered by pH and temperature variations will be discussed. These capsules with predictable shape and size-changing properties hold promise for controlled drug delivery and cellular uptake. Finally, we will address an application of multilayer films in cell-based transplantation therapy for diabetic recipients. We will present a novel type of ultrathin immunomodulatory coating of natural polyphenols and synthetic polymers deposited on surfaces of mammalian pancreatic islets. The coating provides diminished inflammatory immune responses suppressing pro-inflammatory cytokine synthesis and allow for greater enhanced viability and prolonged function of the coated islets.

IBE Abstract # 455

Evaluation of soil organic carbon and soil moisture content from agricultural fields

Environmental Engineering: Ecological and Environmental Modeling

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Abstract

Abstract: Rising CO2 concentrations are worrisome because of the serious threat it poses to global climate. The soil organic carbon (SOC) is strongly affected by human activity and agricultural activity. Few studies have been conducted in the southeastern U.S., particularly Mississippi. This study was conducted in the Town Creek watershed in Mississippi, which evaluated carbon content and soil moisture content of representative soil samples. The soil samples were collected from the cropland fields within the watershed in Mississippi and soil moisture contents and the SOC in the soil profile were measured in the engineering lab.The results of this study demonstrated that the majority of the SOC is contained within the near surface layer of soils studied. There was no statistically significant correlation between carbon content and soil moisture content. These results are reasonable since the amount of the SOC is dependent on the source input of carbon and as well as management practices. Long-term soil and land management datasets, in addition to model estimates, could provide valuable resource information to land managers and policy makers.

IBE Abstract # 488

A Mobile Sensor for Water Quality Monitoring in Water Distribution System

Environmental Engineering: Ecological and Environmental Modeling

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Abstract

A mobile sensor is designed and fabricated for water quality monitoring in the drinking water distribution system. Comparing to the traditional stationary water sensors, a mobile sensor can move with the water flow, which allows it to better locate and provide faster response to a contamination event in the water distribution pipes. An individual mobile sensor device is spherical in shape with a 2.76 inch diameter and can fit most pipe sizes. Half of the sphere contains the Micro-Electro-Chemical-Sensor (MECS) and microfluidics, while the other half contains the electronics. The electronics is completely sealed from moisture, where pogopins provide electrical connection from the MECS to the data-acquisition (DAQ) system. The sensor unit is designed to be released and collected at any water hydrant. This study shows the results of the mobile sensors performance in monitoring various water quality parameters (i.e. pH, water hardness, and disinfectant). Currently, ion-selective membranes are made selective for H+, NH4+, Ca2+, and Mg2+ ions, which are the key ions for pH, water hardness, and disinfectant. The correlation between concentration and electromotive force for the mentioned ions follows the Nernstian equation. This sensor is observed to have linear detection ranges of 10-9 -10-4 M for H+, 10-5.0-10-1.8 M for NH4+, and 10-5.0-10-3.0 M for Ca2+, which covered the concentration ranges of tap drinking water. Ideally, these mobile sensors will act as mobile agents capable of continuously conducting multivariate measurements and reporting them as they are distributed with water pipe flow. The measurement conducted by the mobile sensor is continuous and rapid, with a response time of 15 seconds. The continuous sample tests could simulate real-time tap water flows in the pipe distribution system. Future work will be use the mobile platform for total residual chlorine and hardness change monitoring. Key

words: Mobile Sensor, Drinking Water Quality Monitoring; Micro-Electro-Chemical-Sensor (MECS); Ion-Selective Electrode. Funding support: EPA STAR grant

IBE Abstract # 541

An Ecophysiology Model to Link Genes to Phenotypes of the Common Bean

Environmental Engineering: Ecological and Environmental Modeling

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Abstract

Crop yields can be significantly affected by climate change. Therefore, a major goal of the scientific community is to develop tools that will allow plant breeders to select genotypes that can produce optimal yields under specific environments. These tools will integrate genetic, phenotypic and environmental information into a "gene-based" crop model. As a first step towards building such a tool, a large number (≈ 150) of Recombinant Inbred Lines (RILs) of the common bean (*Phaseolus vulgaris*) along with their parents (Mesoamerican & Andean cultivars) were genotyped using the latest QTL / SNP techniques and grown in various locations (Gainesville, FL; Popayan, Colombia; Palmira, Colombia; Isabela, Puerto Rico; Fargo, ND). The frequency distributions of many traits were characterized in the various environments and showed a wide range of responses. For example, at the cooler, higher elevation site in Popayan the RILs had a range in total leaf area of 140 to 3700 cm^2 at 67 days after planting whereas the warmer location of Palmira had larger total leaf area range of 700 to 7611 cm^2. In contrast, the range on the number of nodes on branches were similar between the two sites at 67 days after planting (5±2 to 70 total nodes on branches) suggesting that this trait may have minimal response to the environment. To link the phenotypic information (e.g., leaf area and number of nodes on branches) to specific genes, the QTLs (Quantitative Trait Loci) controlling growth and development within and across environments will be identified using traditional mapping techniques and a novel method of Functional Mapping; the latter method uses the time-dependent trajectory of a trait to identify QTLs. Also, a model of the early plant growth and branch development of the common bean is being developed to incorporate the genetic information with environmental inputs to predict plant phenotype. Taken together, these efforts will build tools that can be used to select optimal bean genotypes for various environments. Support for this project

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IBE Abstract # 549

Microbial crowd sourcing: Measuring bioavailable nutrient content in soils

Environmental Engineering: Ecological and Environmental Modeling

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Abstract

Effective nutrient management in agricultural and urban settings requires a careful balance between hydrologic events, plant growth, and soil microbiology. Mismanagement of this system can lead to runoff of excess nutrients (nitrogen/phosphorous) and water quality issues in receiving waters. In large part, the activity of microbial communities in soils determines the productivity and overall quality of agricultural and urban ecosystems. These microbes are involved in nutrient cycling, pollutant transformation/detoxification and aggregate stability. Microbial physiology has been used as a sensitive indicator of change(s) in soil water quality for decades, but there are few tools currently available for measuring the fraction of nitrogen/phosphorous which is directly available to soil microbes. Microbial crowd sourcing is a simple, rapid technique for profiling the physiology of native soil communities in the presence of endogenous and supplemented nutrients. The direct measurement of substrate induced respiration (SIR) in a multiplexing format allows rapid profiling; in turn providing large amounts of information about "whole" communities of microorganisms. Soil samples are taken from the field and SIR is monitored in soils with no nutrient and minimally enriched media with ecologically relevant nutrient levels. The difference between endogenous SIR (known as SIRe) and SIR with supplemental nutrients (known as SIRn) provides direct insight into the labile and historical nutrient bioavailable nutrients in the soil. In general, this difference in SIR (known as Î”SIR) decreases following fertilization of agricultural fields. Here, we present the technical details of fabricating and testing the plate assay. We also demonstrate use of the technique for monitoring scrub brush soils, and a field irrigated with hydrolyzed human urine.

IBE Abstract # 429

Nutrient Uptake and Biofilm Formation by Chlorella vulgaris fed with Wastewater

Environmental Engineering: Environmental Complexity and Systems Issues

Yogendra Kanitkar, Dr. Wen Zhang, University of Arkansas

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Abstract

Chlorella sp. are model species of microalgae most widely studied for their nutrient uptake. The idea of using microalgae for nutrient uptake in wastewater is not new to science. However, because of significant cost associated with micro algal harvesting, conventional application remains elusive. Attached growth systems are promising because of the inherent ease in harvesting. But they have been rarely studied for evaluating efficient reactor designs that would aid large-scale commercial applications. The objective of this study was to evaluate the uptake of nitrate and phosphorous and to compare the amount of lipids formed within the attached growth to that of the suspended growth within the reactor. In this study, Chlorella vulgaris was grown in a batch reactor filled with secondary effluent from the West side wastewater treatment plant (Fayetteville, Arkansas) at pH 7.0 and at room temperature (20 ± 2ºC) with artificial illumination (4100 lux). CO2 was supplied using fine bubble diffusers. The culture was regularly wasted and replenished with fresh effluent wastewater from the treatment plant in order to maintain a steady input of nitrate and phosphorous.Results showed that growth rate observed was similar to those documented in the literature. Significant nitrate and phosphate uptake was noted during the growth phase. The attached growth exhibited more lipid formation than that of suspended growth on a dry weight basis. This may be accounted to the formation of exopolymeric substances that anchor the attached growth to the surface. Cell morphologies observed under the microscope were consistent with the Chlorella genus and showed no difference between the attached or the suspended form. The formation of lipids by Chlorella vulgaris can bring an added benefit to its potential large-scale application since recent studies indicate towards their use as biofuels. In addition, biomass accumulated in attached growth can be easily harvested as feedstock for biofuels.

IBE Abstract # 430

Analysis of indirect effects within ecosystem models using pathway-based methodology

Environmental Engineering: Environmental Complexity and Systems Issues

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Abstract

The role of indirect relations within an ecosystem is crucial to its function. Emergent properties such as adaptability, plasticity, and robustness are hard to explain without understanding the system-wide effects of direct and indirect interactions. Two algebraic formulas have been developed to compute the ratio of indirect to direct effects (I/D) in the ecological networks, where I represents indirect relationship and D represents adjacent relationship. In this paper, we take advantage of a different representation of ecosystem models to provide a better understanding of indirect effects. We focus on pathways of individual particles that flow through systems. Particles represent small units of flow material, such as a single carbon atom, 1 g of biomass, or 1 cal of energy. The view of an entire system from an individual particle perspective provides a more practical and intuitive basis to study indirect relations than earlier input-output based algebraic methods. Our findings show that the conventional I/D formulation differs from its intended meaning, which is supposed to compare direct and indirect flows. We come up with a new pathway-based I/D ratio, which revises the current definition, and accurately compares direct and indirect flows. One of the main uses of I/D is to compare ecosystems; however, how the indirect effect is defined and measured might affect the results of analysis. This work has significant impact on past and future studies using indirect effects ratio as a measure for system analysis and comparison. I/D, proposed for evaluating ecological networks, is potentially applicable to analyze systems in other areas.

IBE Abstract # 446

The impact of continued nutrient enrichments on disinfection byproduct formation

Environmental Engineering: Environmental Complexity and Systems Issues

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Abstract

Disinfection byproducts (DBPs) are formed by reactions between disinfectants (e.g., free chlorine) and natural organic matter (NOM), which is ubiquitous in lakes and rivers. Higher concentrations of DBPs are formed in the summer months, when algal communities are more active. Continued nutrient enrichment of nitrogen and phosphorus are expected to increase primary productivity and shift the phytoplankton community composition to predominately cyanobacteria in both suspended and sessile growth. However, the impacts of nutrient enrichment on NOM properties and DBP formation are not well understood. In this study, a series of nutrient enrichment mesocosms with Beaver Lake water (Lowell, Arkansas) were used to investigate the impact of phytoplankton biomass on physiochemical NOM properties and DBP formation and control. Algal biomass was quantified using a coulter counter and fluorescence microscopy; NOM was characterized physically by asymmetric flow-field flow fractionation (AF4) and chemically by fluorescence excitation-emission matrices. DBP formation potential tests with free chlorine showed increased algal biomass with increasing phosphorus loadings contributed to increase formation of trihalomethanes and haloacetonitriles. The results of this study will be of vital importance to drinking water treatment plants to develop strategies to meet current and pending DBP regulations.

Synthesis of formate through reduction of CO2 catalyzed by acidophilic formate dehydrogenase

Environmental Engineering: Environmental Complexity and Systems Issues

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Reducing the atmospheric CO_2 level has received much attention recently as an approach to combat global warming, but this process has remained a challenge. Enzymatic CO_2 reduction has been examined extensively as a promising approach to greenhouse gas fixation and the production of renewable fuels and chemicals. Nicotinamide adenine dinucleotide (NAD)-dependent formate dehydrogenase from *Candida boidinii* (CbFDH) is the most popular CO_2-reducing biocatalyst, but its activity is too low to allow efficient CO_2reduction. In this study, we investigated the feasibility of using acidophilic formate dehydrogenase (FDH) as a CO_2-reducing biocatalyst. Five acidophilic FDHs were selected based on biochemical data, and four FDHs exhibited greater CO_2 reduction activity than CbFDH. The FDH from *Thiobacillus* sp. strain (TsFDH), which had the highest CO_2 reduction activity, had a dramatic preference for the reduction reaction (with a 55.4-fold higher ratio of CO_2 reduction to formate oxidation for the catalytic efficiency (k_{cat}/K_B)) relative to CbFDH. In an electrochemical reactor in which NADH was regenerated, the use of TsFDH resulted in an 87-fold higher formate production rate than commercial CbFDH. These experimental results demonstrate that TsFDH can be an alternative to CbFDH as the biocatalyst in CO_2 reduction systems.

IBE Abstract # 494

Microbial Biofilm Proton and Oxygen Flux during Biogenic Corrosion of Cement

Environmental Engineering: Environmental Complexity and Systems Issues

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Abstract

Microbially induced corrosion (MIC) by sulfuric acid-producing bacteria is one of the main sources of concrete deterioration. The low durability of some types of concrete infrastructure indicates that current mixture design based on concrete specifications may not be appropriate to address the aggressive nature of wastewater. Cement paste samples of six different mixtures were designed to determine the influence of mixture proportions on the rate of deterioration and identify the role of each component. Mature Thiobacillus sp. biofilm was developed on sample surfaces and a noninvasive, micro sensor was used to quantify microbial metabolism and their interaction with cement through real time determination of oxygen and proton flux changes, respectively. Through detailed examination of typical mixture designs under extreme environmental conditions, higher water/cement ratio resulted in more bacteria growth and biofilm development, and more biofilm activities. The pozzolanic reaction during cement hydration with different components had great impact on the biofilm activities as well. Biofilm stress response to common environmental toxins in wastewater was also characterized, showing significant O2 and H+ flux increase. The biofilm characteristics determined by the real time O2 and H+ flux combined with cement mixtures analysis could improve our understanding of the biological deterioration mechanisms associated with the critical infrastructure application in wastewater transport and treatment.

IBE Abstract # 495

Assessing differences in mechanism of toxicity of ionic silver and silver nanoparticles in D. manga embryos

Environmental Engineering: Environmental Nanotoxicology

Matthew C Stensberg (Presenting), Purdue University; Rajtarun Madangopal, Purdue University; Qingshan Wei, Purdue University; Gowri Yale, Purdue University; Hugo Ochoa-Acuna, Purdue University; Alex Wei, Purdue University; Eric McLamore, University of Florida

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Abstract

Silver nanoparticles (AgNPs) have been incorporated, to date, in over 1000 products, primarily for their use as antimicrobial agents; however, the mechanisms of toxicity of AgNPs to other organisms are not fully understood. Many traditional toxicological studies solely rely on invasive or destructive techniques to evaluate this. It is the goal of this research to determine the difference in physiological effects induced by ionic silver (as silver nitrate, AgNO3) and AgNPs in the water flea, Daphnia magna, while incorporating non-invasive toxicological methods. D. magna embryos (stages 1-2) were exposed to sublethal doses (130 ng/L and 650 ng/L) of AgNO3 and AgNPs. AgNP uptake was confirmed using confocal reflectance microscopy. Differential imaging was used to track embryonic development and showed that, at low levels, ionic silver had a greater inhibitory effect on growth. ICP-MS was used to measure embryonic sodium concentration (loss of this ion is a known response of ionic silver toxicity). While the general sodium displacement was similar for both ionic silver and AgNPs, the silver loading for AgNPs was significantly greater than ionic silver, implying the ionic form is a more efficient displacer of sodium. Mitochondrial function was non-invasively monitored by tracking changes in proton flux using self-referencing microsensors. Flux measurements showed that while both forms of silver significantly affected proton efflux, the change induced by AgNPs was significantly greater than that of ionic silver. The fluorescent dye, JC-1, an indicator of mitochondrial permeability, was used to independently confirm these results. Thus, while both ionic silver and AgNPs affected mitochondrial function and total internal sodium concentration in D. magna embryos, the magnitude of these effects was not consistent for both

forms of silver across these toxicities. Taken together, these results imply that the mechanisms of toxicity of ionic silver are different from those of AgNPs.

IBE Abstract # 547

Decentralized Graywater Recovery using Bioreactors: Effects of Household-derived Silver Nanoparticles

Environmental Engineering: Environmental Nanotoxicology

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Abstract

One solution to reducing water demand and alleviating pressure on depleting municipal water supply is use of decentralized resource recovery technologies for recovering useable water from wastewater. One of the largest household wastestreams is graywater (GW), which makes up over half of household wastewater (by volume). Direct reuse of GW for outdoor (e.g., irrigation) and indoor purposes (e.g., toilet flushing) represents a sustainable solution to reducing household water consumption; currently, these water uses consume drinking water in most households. Current physicochemical GW treatment methods for decentralized reuse are efficient, but these technologies are resource intensive and require significant maintenance. Bioregenerative technologies such as microbial bioreactors are a sustainable alternative option which are self-maintaining and require fewer consumables than physicochemical techniques. Bioreactors for treating GW utilize the metabolic activity of acclimated bacterial cultures, and current approaches focus on use of biofilm reactors due to enhanced resilience during changes in operating conditions. However, emerging contaminants such as antimicrobial silver nanoparticles (SNP) commonly found in household wastewater would be detrimental to beneficial microbes designed to process GW. This research demonstrates use of a hollow fiber membrane-aerated bioreactor (HfMBR) for treating household GW, and investigates the effect(s) of SNP on microbial physiology. HfMBR effluent water quality was analyzed using common wet chemistry techniques; bulk liquid carbon turnover, and effluent pH/O2 significantly decreased immediately after exposure, although the treatment efficiency recovered within 2-3 days. Scanning electron microscopy and transmission electron microscopy were used to visually inspect SNP adhesion to the biofilm surface, and electron dispersive X-Ray elemental analysis

confirmed the presence of high Ag+ concentrations in nanoparticles. Physiological flux studies using self referencing microsensors indicated acute uncoupling of oxidative phosphorylation and disruption of proton homeostasis, but gradual recovery within approximately 1 hour. In addition to these changes in microbial metabolism, exposure to SNP caused immediate detachment of sessile cells and exopolymers from membrane-immobilized biofilms. Ongoing studies are examining he effect(s) of acute and chronic SNP exposure to beneficial microbes processing household GW.

IBE Abstract # 438

Optimization of Biofilms for Bioreactors

iGEM Synthetic Biology

Sean Michael Kearney, Purdue University; Amanda Shanley, Purdue University; August Clevenger, Purdue University; Rajtarun Madangopal, Purdue University; Kari Clase, Purdue University; Jenna Rickus, Purdue University

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Abstract

Graywater, water originating from laundry, dishwashing, or bathing, can be recycled and reused in residential settings as a non-potable water source. Treatment of this water by microbial digestion provides one route to make it more amenable for storage and later use. Reactors used in microbial digestion of graywater sources often use communities of microorganisms formed in bioflms in membrane-aerated bioreactors. Use of these biofilms suffer from two technical challenges - start-up time (flow-through of microorganisms prevents the rapid establishment of biofilm communities), and fouling (detachment of microorganisms blockades the bioreactor, preventing efficient digestion). We developed a model system in which we could influence one particular species of bacteria (E. coli) to form biofilms more rapidly and to reduce detachment once formed on a surface. At the genetic level, we designed two devices. The first produces membrane-bound proteins for cell-cell and cell-surface adhesion. The other yields membrane-attached fusion proteins that bind silica, forming a continuous silica matrix over the biofilm and protecting the cells from shear-induced detachment and damage. We have shown that the adhesion proteins accelerate the rate of biofilm formation in static and continuous flow environments and that silica matrices successfully form on cell membranes.

IBE Abstract # 476

Detection of water-borne pathogens via split beta-galactosidase complementation

iGEM Synthetic Biology

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Abstract

Bacterial pathogens including E. coli O157:H7 serotype, Campylobacter, Shigella, and Salmonella often contaminate drinking water supplies in developing nations. These microbes are responsible for approximately two million worldwide annual deaths via diarrhea. Current technologies for detection of bacteria include DNA hybridization FRET signaling, electrical detection, and PCR amplification followed by gel visualization. Multiple barriers prevent effective use of these current technologies in the field. We aim to make a rapid, low cost, highly portable biosensor by producing enzymatic detector molecules in an easy-to-culture cell strain. Our biosensor design consists of a modular single stranded DNA domain for binding specific pathogenic DNA, a protein-DNA linker domain, specifically D168A topoisomerase, and a split beta galactosidase. Target probe sequences were designed to complement sequences of genomic DNA twenty base pairs apart on a particular pathogen. Incubating our fusion protein biosensor with target probe sequences flanked by recognition sites can produce covalent DNA-protein complexes that can be subsequently purified. When several biosensor molecules are brought close together via pathogen DNA binding, a functional beta-galactosidase unit is formed from the split fragments and produces a colorimetric output. Preliminary data, verified via mass spectrometry and protein quantification assays, indicate that we have been able to successfully express and isolate individual functional proteins in our design. D168A topoisomerase has the ability to covalently bind to specific target sequences on a template plasmid. In addition, experimental data shows that split beta-galactosidase is effective as a robust reporter system for water testing. Our research demonstrates success in initial stages of chimeric protein assembly.

IBE Abstract # 478

Arachnicoli: Production and Purification of Spider Silk Proteins in Escherichia coli

iGEM Synthetic Biology

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Abstract

Spider silk is a biomaterial with extraordinary physical properties. The combination of tensile strength, elasticity, and even biocompatibility has opened eyes to a wide range of potential applications. A few implementations of spider silk may one day include: medical advances (skin grafts, biomedical sutures, and artificial tendons/ligaments), automotive safety (seat belts and airbags), and military applications (parachutes and body armor). However, the production of spider silk is not as simple as merely farming spiders and collecting the silk. Spiders are very territorial and cannibalistic, thus alternative means of production are necessary to generate enough spider silk for realistic use. The bacterium Escherichia Coli has been proven effective at producing this valuable product because of its manipulability and our intimate knowledge of the organism's genome and functions. Through the use of synthetic biology and molecular cloning techniques, recombinant DNA has been constructed and transformed into E. coli for production of synthetic spider silk. The goal is to take advantage of E. coli's ability to be used as a 'factory' for creating silk in a controllable and cost efficient system. Supplementation with additional tRNAs will be employed as a strategy to boost overall silk yield and extend cell viability. The creation of longer, repetitive spider silk genes will allow for a broad range of 'designer' silk to be synthesized and subsequently tested for increased mechanical properties. The spider silk gene sequence of the Argiope aurantia has been chosen because of the physical properties it displays as well as the relative lack of extensive studies.This year, the Utah State iGEM team has constructed the first spider silk BioBrick parts. A composite system was created from these parts, which allowed for the production of the spider silk protein in E. coli. After extracting and purifying this protein, synthetic spider silk was artificially spun

into fibers. From the constructed parts, 16 highly characterized genetic components were sent to the Registry of Standard Biological Parts. Over 64 BioBrick parts were assembled and all of these are available upon request.

IBE Abstract # 548

Applying innovations in Human-Computer Interaction for Supporting Discovery and Learning in Synthetic Biology

iGEM Synthetic Biology

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Abstract

Over the last two years, the Wellesley Human-Computer Interaction (HCI) Lab has designed a variety of software applications that tackle the question of how innovations in human-computer interaction can enhance learning and discovery in synthetic biology. In the field of synthetic biology, designing complex biological systems and processing related metadata is becoming increasingly important. However, there is still a deficit of computational tools that streamline the research workflow for synthetic biology researchers. Through interdisciplinary research that combines rigorous user-centered and participatory design methods we developed three distinct tools for synthetic biology- G-nome Surfer Pro (2011), MoClo Planner (2012), and SynBio Search (2012). These tools encourage collaborative research, simplify information gathering and support troubleshooting through intuitive multi-touch user interfaces. G-nome Surfer Pro is a tabletop user interface that provides an integrated environment for viewing prokaryotic genomic data and literature accessed from Genbank and PubMed. G-nome Surfer was created by the Wellesley-BU iGEM 2011 team. MoClo Planner, is a collaborative, large-scale multi-touch software tool that visualizes and simplifies the design of multi-gene constructs using Golden Gate Modular Cloning. Synbio Search, is a semantic web search tool which creates a comprehensive data sheet for over 2700 biobrick parts by integrating and linking information from various data sources, including the MIT Registry of Standard Biological Parts, iGEM Archive, Google Scholar, and PubMed. The MoClo Planner and SynbioSearch were created by the Wellesley iGEM 2012 team in collaboration with the BU and MIT iGEM teams, and for the MoClo Planner, also the BU CIDAR group and Agilent Technologies.We tested the usability and usefulness of these software tools with undergraduate biology, biochemistry, and

bioengineering students. Users found the software tools to be intuitive and engaging. We also found that our software tools lower the threshold for using advanced functionality and for understanding complex synthetic biology systems. Our projects won Best Software Tool (2011) and Best Requirement Engineering (2012), Best SBOL-Implementation (2012), and Best Eugene-Based Tool (2012) at iGEM World Championships. We hope to continue and develop, in collaboration with the community, novel software tools that enhance discovery and learning in synthetic biology.

Real-time quantitative measurement of RNA and protein levels using fluorogen-activating biosensors

iGEM Synthetic Biology

Eric Pederson (presenting), Carnegie Mellon University; Yang Choo, Carnegie Mellon University; Peter Wei, Carnegie Mellon University; Jesse Salazar, Carnegie Mellon University; Cheemeng Tan, Carnegie Mellon University; Natasa Miskov-Zivanov, Carnegie Mell, Electrical and Computer Engineering

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Abstract

The design and implementation of synthetic biological systems often require quantitative information on both transcription and translation rates. However, quantitative information about the expression strength of a synthetic promoter has been difficult to obtain due to the lack of noninvasive and real-time approaches for measuring the levels of both RNA and protein in cells. Here, we engineer a fluorogen-activating biosensor that can provide information on both transcription strength and translation efficiency. The biosensor consists of an RNA aptamer called Spinach and a fluorogen-activating protein (FAP). This biosensor is non-invasive, easily applied to a variety of promoters, and more efficient than existing technologies. Specifically, Spinach is fused to the un-translated region (UTR) of an mRNA as a RNA reporter and FAP is expressed as a protein reporter. Fluorogenic dyes DFHBI and malachite green are non-fluorescent in solution, but fluoresce brightly when bound to Spinach and FAP, respectively. This way, the biosensor allows us to determine the concentrations of specifically tagged RNA and protein. To demonstrate the utility of our biosensor, we constructed and characterized several designed T7Lac hybrid promoters. Furthermore, we developed a mathematical model of our synthetic system to guide experiments and interpret data. Based on both mathematical modeling and experimental studies, we found that our promoters behaved as expected: the promoters designed to have lower initiation frequency or lower binding affinity to T7 RNA polymerase produced less mRNA and protein, while promoters designed to have higher binding affinity to T7 RNAP produced higher concentrations of mRNA and protein. To expand our efforts in human practices and education, we developed an open-source electronic kit that mimics our experimental setup. The electronic kit complies with the Pennsylvania educational requirements to lower the barrier for adoption by teachers in high school classrooms. After the 2012 iGEM competition, where we received awards for “Best Experimental Measurement Approach” and

"Best Foundational Advance", we continue to improve both robustness and modularity of the biosensor. Our work would have implications on the standardization of synthetic biological parts and the characterization of host-circuit interactions.

IBE Abstract # 567

Do Multiple Start Codons Affect Codon Slippage?

iGEM Synthetic Biology

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Abstract

The central dogma of molecular biology dictates that DNA makes RNA makes protein, but the dogma does not always accurately predict results observed in biology laboratories. Especially in the field of synthetic biology, understanding the special cases and functional anomalies of DNA can streamline design and synthesis. In this research, the possibility of multiple start codons and their effect on codon slippage is observed and measured using a synthesized DNA construct. The phenomenon of codon slippage can result in the production of unexpected proteins, and warrants further exploration and characterization. A plasmid construct was rationally designed to investigate the effects of multiple start codons on codon slippage during translation. Using traditional cloning techniques, the sequence was constructed to test the selection of start codons by the ribosome during translation by manipulating the DNA sequence to include two start codons in different open reading frames. The start codons are separated by a number of nucleotides which place them in distinct open reading frames. Each start codon corresponds to a fluorescent protein reporter within the start codon's respective open reading frame. Thus, the selection of the first start codon, second start codon, or a combination of both during translation results in the expression of a fluorescent protein in the corresponding open reading frame. The construct was synthesized using annealed single-stranded DNA sequences with 40 basepair overhangs corresponding to adjacent sequence overhangs. The incidence of start codon slippage from one to the other start codon is quantified by measuring the relative expression of each fluorescent reporter. Codon slippage occurred in start codons separated by a seven nucleotide sequence. Thus, multiple start codons within a DNA sequence can affect the expression of protein.

IBE Abstract # 568

Genetically engineered bacteriophage for diagnosis of whooping cough

iGEM Synthetic Biology

Joshua Fass, University of Virginia (Corresponding and Presenting Author); Jacqueline Grimm, University of Virginia; Syed Hassan, University of Virginia; John Hubczak, University of Virginia; Shaun Moshasha, University of Virginia; Joseph Muldoon, University of Virginia

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Abstract

Whooping cough, the infectious respiratory disease caused by Bordetella pertussis, is diagnosed in tens of millions of people and results in almost 300,000 deaths globally each year. Low-income and unvaccinated individuals as well as infants are especially susceptible. Current diagnostic procedures are complicated, costly, and can take up to a week, by which time the disease may have progressed or spread. The enormous impact of this disease urgently motivates the development of a faster, cheaper, and more reliable diagnostic. Our epidemiology models suggest that earlier diagnosis could drastically reduce the incidence and impact of the disease. We propose an engineered bacteriophage diagnostic system for rapid clinical detection of pertussis. As a proof of principle, we worked on engineering the T7 bacteriophage to express a version of the beta subunit of human chorionic gonadotropin (hCG) which we codon-optimized for expression in E. coli. Due to host-pathogen specificity, bacteriophages will only replicate and produce intracellular hCG if the target bacteria is present in the sample. The bacteriophage lyses the cell, releasing the hCG, which can then be detected using a pregnancy test. Pregnancy tests are commonly available in all clinics and can detect very low concentrations of hCG, which is an advantage over currently-available methods for detecting phage amplification. The bacteriophage system also requires minimal training to be used in lab and does not require any high-tech machinery. This will make the diagnostic readily usable in developing countries, where whooping cough is a particular concern. Beyond B. pertussis, this method can potentially be used to detect the presence of any bacterium for which there exists a bacteriophage using a common pregnancy test.

IBE Abstract # 431

Sugar Utilization in Escherichia coli at the Single-cell Level

Metabolic Pathway Engineering

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Abstract

Microorganisms can be metabolically engineered to convert renewable feedstocks into biofuels, fine chemicals, and pharmaceutical drugs. In most metabolic engineering applications, chemical production begins with the uptake and utilization of simple sugars. Microorganisms such as Escherichia coli encode numerous sugar utilization pathways that are induced only in the presence of the sugar. Developing a comprehensive understanding of these pathways is an essential step toward engineering efficient chemical production. While sugar utilization pathways have been extensively studied over the past 60 years, the vast majority of studies employed bulk characterization techniques that miss complex behaviors in single cells. The few single-cell studies revealed that a sugar either negligibly or fully induces the pathway - what has been called the 'all-or-none' response. This response would pose a bottleneck in metabolic engineering by inactivating a portion of the microbial population, thereby reducing product conversion rates.One critical feature of the previous single-cell studies was a lack of sugar utilization, either through the use of a non-hydrolyzable inducer or through the deletion of the catabolic genes. In contrast, natural utilization pathways inherently consume the inducing sugar. To address this disconnect, we have been examining the single-cell response of sugar utilization pathways in E. coli using both experimental and computational approaches. In this talk, we will discuss our characterization efforts of the L-arabinose utilization pathway in E. coli. Using flow cytometry analysis, we observed that the pathway exhibits a combination of graded and 'all-or-none' responses - what we call a 'some-or-none' response. Deletion of the L-arabinose catabolic genes restored the classic 'all-or-none' response. Remarkably, the 'some-or-none' response lacked hysteresis and appeared to reflect regular switching between induced and induced states, where a stochastic model made similar predictions. Overall, our findings will redefine our

understanding of sugar utilization in microorganisms and will provide significant insights into engineering strains with homogeneous and predictable behavior.

IBE Abstract # 508

Characterization of the Pradimicin A Biosynthetic Pathway

Metabolic pathway engineering

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Abstract

Pradimicin A is a potent antifungal and antiviral natural product. The late biosynthetic steps in pradimicin biosynthesis are not well understood. In this study, we investigated the pradimicin biosynthetic pathway through combinatorial biosynthesis and gene disruption approaches. We have obtained four new pradimicin biosynthetic intermediates, KN92, KN90, KN87, and KN82, which for the first time confirmed the functions of pdmW and pdmS and revealed the order of four important tailoring reactions in the pradimicin biosynthetic pathway. This work allows us to understand pradimicin biosynthesis and further engineer the biosynthesis of new pradimicin analogs with improved therapeutic characteristics.

IBE Abstract # 456

Metabolic flux redistribution for enhanced production of 1,2-propanediol and 1-propanol in Escherichia coli

Metabolic Pathway Engineering

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Abstract

The depletion of fossil fuels poses as a threat not only to the environment but also to a number of petrochemical products. The release of green house gas carbon dioxide plays a crucial role in global warming and soon it will be vital to rely on an alternative source of fuel. It is also predicted that due to the rapid consumption of fossil fuels the cost of manufacturing petrochemicals will rise dramatically. 1,2-propanediol is an example of a commodity chemical being produced from oil and 1-propanol is an example of a potential biofuel manufactured from oil. In this work we have pursued the biological manufacture of 1,2-propanediol and 1-propanol using glucose in Escherichia coli. The tools of metabolic engineering and synthetic biology allow researchers to develop novel synthetic pathways. However, the establishment of a pathway by itself does not provide a solution. In order for a biological process to be commercially viable a high production level and yield is necessary. In this work we exhibit the systematic improvement in production levels and yield of 1,2-propanediol and 1-propanol. In order to do so we established a novel synthetic route for production by engineering the glycolytic pathway. We then achieved the systematic redistribution of carbon flux to increase the yield. Following this we exhibit the manipulation of cellular energetics to boost the production of 1,2-propanediol at over 2g/L in shake flask studies. We also report a product yield of >80% of theoretical maximum from our engineered strains. By optimizing protein expression levels we improved 1-propanol production over 6 times. This work represents the highest titer and yield achieved in shake flask studies for 1,2-propanediol and 1-propanol production and represents the efforts of metabolic engineers in expanding natural metabolism for the production of green chemicals.

IBE Abstract # 469

Host selection for synthetic pathways using a computational systems biology approach to explore biodiversity

Metabolic Pathway Engineering

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Abstract

Systems metabolic engineering is growing rapidly towards enabling strategies to engineer new and improved phenotypes. An important focus in this field is on engineering strains capable of producing chemicals and fuels using non-natural biochemical routes in different hosts. In this regard, the majority of focus has been on well-studied organisms such as E. coli, B. subtilis, and yeast. Given the exponential growth of '-omics' data and the rapid advancements in molecular biology, our knowledge is becoming enriched on the physiology and biochemistry of lesser-studied organisms. These organisms may ultimately be found to be superior for the production of fuels and chemicals. A crucial challenge herein is the selection of an optimal host among several preferred candidates that all remain lesser-studied. To achieve this task, we have developed a computational framework for quick and comprehensive scanning of biodiversity to select an optimal host for the production of target chemical given a de novo biosynthetic pathway(s). This method makes use of several genome-scale metabolic flux models to predict the production of non-native chemical(s) in silico by selected organisms. Combining the high-throughput '-omics' data with available physiological and biochemical information, genome-scale models have been shown to be powerful tools for studying cellular metabolism at a system level. However, genome-scale models are commonly written using different formats and, therefore, standards between them were established in order to be able to compare multiple models side-by-side. To develop these standards, we created a novel algorithm that draws associations between metabolites of different models (and therefore, the metabolic networks of models). Then, we created a database of the standardized models through indexing metabolites and metabolic reactions. This database of genome-scale models allows the user to incorporate user-defined synthetic pathways to any model and retrieve a modified model in Systems Biology Markup Language (SBML) format. A web-interface application was developed in PHP language

to serve as a platform for synthetic pathway addition to genome-scale models with importance in biotechnology and further share the models with other researchers. The computational method developed in this study can be considered as a priori to synthetic biology where time-consuming resource-intensive expensive instrumentation is required. Several working examples of our new database system for host selection will be presented in detail.

Deriving Metabolic Engineering Strategies with Flux Ratios Genome-Scale Modeling

Metabolic Pathway Engineering

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Abstract

A new method of using flux balance analysis with flux ratios (FBrAtio) is fully described in this presentation and is applied to five case studies to evaluate and design metabolic engineering strategies for microbial cell factories. Ultimately, this approach will be used to drive systems metabolic engineering, where genome-scale metabolic models and computational tools are used to design metabolic engineering strategies, which are then implemented and optimized by the experimentalist. The FBrAtio methodology was implemented on case studies derived from publicly available genome-scale metabolic flux models. Synthetic pathways were added to these models along with flux ratio constraints by FBrAtio to achieve increased (i) cellulose production from *Arabidopsis*; (ii) isobutanol production from yeast; (iii) acetone production from *Synechocystis* sp. PCC6803; (iv) H_2 production from *Escherichia coli* MG1655; and (v) isopropanol, butanol, and ethanol (IBE) production from engineered *Clostridium acetobutylicum*. The FBrAtio approach was applied to each case study to simulate a metabolic engineering strategy already implemented experimentally. Flux ratios constraints were adjusted in each case to find (i) the end-limit of increased production using the existing strategy, (ii) new potential strategies to increase production, and (iii) the impact of these metabolic engineering strategies on product yield and culture growth. While the FBrAtio approach can be used in this manner to derived "fine-tuned" metabolic engineering strategies, several challenges still exist. Several of these are detailed in the presentation and are evident in a sensitivity analysis performed around constraining several flux ratios in a highly-interconnected network of clostridial central carbon metabolism.

IBE Abstract # 502

Pathway Pioneer: A Web-based Network Visualization and Flux Analysis Tool

Metabolic Pathway Engineering

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Abstract

As stoichiometric metabolic models increase in complexity and fidelity, design and reconstruction tools are urgently needed to increase the productivity of this time-consuming process. Engineers require software for the exploration and rapid analysis of design alternatives, integrated with intuitive network visualization. This work introduces such a tool: PathwayPioneer (www.pathwaypioneer.com), a dynamic web-based system build as a front-end graphical user interface to the well-established flux balance analysis tool COBRA. Pathway Pioneer adds additional functionality for model-engineering collaboration through shared models and model version control. Pathway Pioneer is a dynamic, clickable browser-based visualization system for metabolic network models retrieved from databases such as BiGG or developed in-house as SBML or XLS compliant files. The network is displayed by the browser using existing map coordinate files, if available, or using built-in layout algorithms. The tool supports many network-level operations including zooming and panning, level-of-detail control, flux visualization, keyword searching, and hierarchical subsystem organization. A reaction may be knocked out, set as an objective, looked up in a database or many other operations by a single click. Following each operation the visualization is refreshed with the new flux values. To support understanding as networks are reconstructed and extended graph rearrangement is minimized as reactions are added or changed. The system supports model revision control to manage alternative network configurations and allows roll-back to earlier versions and the merging of branched models.Pathway Pioneer has been applied to many standard models and is currently under user testing for new models under reconstruction in the Cho system and others.

IBE Abstract # 509

Regulation of the production of antitumor chromomycins in Streptomyces roseiscleroticus

Metabolic Pathway Engineering

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Abstract

The whole genome of Streptomyces roseiscleroticus (ATCC 53903) has been recently sequenced by our group. A putative type II polyketide biosynthetic gene cluster was discovered and its function has been predicted to be involved in the biosynthesis of chromomycins, a group of potent anticancer natural products. Under laboratory conditions, this gene cluster is silent. Two regulatory genes were manipulated to turn on the biosynthetic pathway, which led to high yield production of these anticancer drugs in the engineering strain. Therefore, our work provides useful metabolic engineering tools to turn on silent gene clusters and enhance the yield of target compounds through the regulatory genes.

Raman spectroscopy for metabolic engineering applications

Metabolic Pathway Engineering

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Abstract

Metabolically engineered cells often exhibit phenotypic characteristics, including an overall chemical composition, vastly different from their wild-type counterparts. Characterization of these phenotypes has been costly and time-consuming, but new developments with Raman spectroscopy are providing near-instantaneous measurements of cell chemical composition. In particular, Raman spectroscopy is a powerful bianalytical tool that provides an attractive method for metabolic analysis of cellular systems because it is nondestructive, label-free, and can be conducted in real time. However, Raman spectroscopy is yet to realize its promised potential in biotechnology research primarily because Raman spectra of complex biological systems are difficult to interpret. A Raman spectrum of a microorganism is in essence an image that captures its entire chemical composition in a single spectral image. Extracting useful metabolic information from this 'image' remains the real challenge. In recent research, we have developed experimental and chemometric procedures that facilitate the application of Raman spectroscopy in metabolic engineering research. In the first example, the utility of Raman spectroscopy is demonstrated to monitor metabolic changes in *E coli* under 1-butanol stress. For instance, results show that Raman spectroscopy was as effective as gas chromatography–mass spectrometry in determining saturated to unsaturated fatty acids ratio; an important indicator of microbial adaptation to toxic alcohols. In another study, Raman spectroscopy is used to create a reference phenotypic database of *E coli* treated by antibiotics with known mechanisms of action. Our preliminary results show that this database can be useful in determining the molecular targets of a newly developed antibiotic. The detection of molecular targets of a putative compound remains the bottleneck in drug discovery. Furthermore, we developed novel peptide-guided silver nanoparticle probes for quantitative surface-enhanced Raman scattering (pgSERS probes). The pgSERS approach leverages the remarkable sensitivity of SERS while significantly eliminates the associated reproducibility problem through selective localization of the

pgSERS probes based on the amino acid sequence of the peptides used. For example, with a membrane targeting pgSERS probe, it was possible to selectively analyze the outer membrane of *E coli* and obtain *in vivo* quantitative metabolic measurements.

IBE Abstract # 407

Multiplexed detection of biomarkers using phase change nanoparticles

Nanomaterials and Nanosystems

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Abstract

A new biological sensing method based on phase change nanoparticles has been developed for highly sensitive and multiplexed detection of multiple protein biomarkers. A panel of solid to liquid phase change nanoparticles with composition controlled melting temeprature have been designed, synthesized, and modified with a panel of antibodies or single strand DNA to form a one-to-one accordance between each type of nanoparticles and each type of biomarkers. A solid substrate will be co-modified with a mixture of antibodies, and used to bind biomarkers from solution, which is followed by attracting antibody-attached nanoparticles. After removing unbound nanoparticles, those attached on substrate will be readout using differential scanning calorimetry (DSC), in which the melting peak and melting enthalpy will be used to determine the type and the concentration of according biomarkers. By detecting multiple biomarkers from the same sample, multiple species can be detected within a minimal amount of sample.

IBE Abstract # 412

Top-down fabrication of particulate micro/nanodevices for drug delivery and cell tracking

Nanomaterials and Nanosystems

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Abstract

Top-down fabrication technology allows production of devices with precisely controlled and unique structures at the micrometer and nanometer scales. We have developed a novel top-down fabrication technique by integrating layer-by-layer assembly and microcontact printing for producing particulate micro/nanodevices for drug delivery and cell tracking applications. The technique is highly versatile in producing micro/nanodevices with various shapes, sizes and structures. A variety of functional materials can also be incorporated into the devices including polyelectrolytes, thermoplastic, and nanoparticles. In particular, we have fabricated microdevices composed biodegradable polyelectrolytes and thermoplastic organized in a unique dot-on-pad structure, which may allow unidirectional drug delivery to single cells. Moreover, we have built multilayered microdevices containing both gold nanoparticles and Raman reporter molecules, which can generate strong Raman scattering signals and potentially allow in vivo cell tracking using near infrared light.

IBE Abstract # 416

Micellar nanodroplet-assisted ligand exchange of metal complex by dsDNA

Nanomaterials and Nanosystems

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Abstract

Study of the interactions between metal complexes and biological targets such as DNA has attracted a vivid interest in the field of medical diagnostics. Most current detection methods for the sequence specific recognition of DNA make use of the unparalleled hybridization property of ssDNA to base-pair with high specificity to a complementary DNA strand. Despite considerable advances in the DNA detection via hybridization, few studies have been reported for direct detection of dsDNA that circumvents the need for denaturation and subsequent hybridization. The ultimate goal of this research is to develop a novel colorimetric sensing strategy for DNA detection, employing the competitive binding of gallium (III) ions to DNA and 4-(2-pyridylazo) resorcinol (PAR)-modified gold nanoparticles. The effect of micelles of different charge types on the ligand exchange reaction between Ga (III)-PAR complex and calf thymus DNA was studied spectrophotometrically, under varying buffer, pH, and ionic strength conditions. Micelles such as sodium dodecyl sulfate (SDS) and cetyltrimethylammmnium bromide (CTAB) markedly enhanced the intensity of Ga(III)-PAR complex. Moreover, the results reveal a striking selectivity for the ligand exchange of Ga(III)-PAR by calf thymus dsDNA over calf thymus ssDNA. Coupled with gold nanoparticle-based nanoplasmonic colorimetry, this unique sensing mechanism has the potential to become a simple and cost-effective modality for point-of-care testing of genetic biomarkers, particularly for infectious diseases.

IBE Abstract # 444

Carbon Nanotubes Interfacing Bacillus anthracis Spores

Nanomaterials and Nanosystems

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Abstract

Carbon nanotubes (CNTs), as major building blocks of the newly emerged nanotechnology, have captured a great deal of attention in research since their discovery in the early nineties due to their unique optical, electrical, mechanical, and thermal properties. There has been enormous interest in exploiting their remarkable properties for numerous biological applications. Many of the studies have been focused on human cells. Not until the past three years, studies have been extended to CNTs' interactions with bacterial cells. Our group is exploring the antimicrobial activities of single-walled carbon nanotubes (SWNTs) to B. anthracis spores. We studied the effect of SWNTs suspension on inactivation of B. anthracis spores and biofilm formation, the effects of SWNTs coupled with oxidizing chemicals or near infrared (NIR) radiation on inactivation of spores, as well as spore attachment on different types CNTs-modified surfaces (CNT forest and CNT sheet). We found that SWNTs alone did not effectively inactivate B. anthracis spores at concentration < 250 μg/ml, while SWNTs (100 μg/mL) coupled with H2O2 (1.5%) or NaOCl (0.25%) had the synergistic effect contributed by the two individual antimicrobial mechanisms of SWCNTs and the oxidizing antimicrobial chemicals, and resulted in much stronger sporicidal effect compared to treatment with H2O2 or NaOCl alone at the same concentrations, doubling the log reduction of viable spore number (~3.3 log vs. ~1.6 log). SWNTs coupled with NIR had dual effect on B. anthracis spores. It enhanced the sporicidal effect but stimulated the germination of surviving spores at the same time. Multi-walled carbon nanotubes (MWNTs) modified surfaces, including MWNT forest on silicon wafer and MWNT sheet on poly(methyl methacrylate) (PMMA) film, significantly increased surface hydrophobicity ($P<0.05$) and enhanced the attachment of spores on their surfaces compared to the uncoated substrates, respectively, showing their potential as adsorbents for removal of Bacillus spores from fluids. However, no inhibitory effect was observed on the germination of attached spores on both types of MWCNT surfaces. The interactions between CNTs and B. anthracis

spores at various fore mentioned conditions were studied using fluorescence and electron scanning microscopes.

KEYWORDS: Carbon Nanotubes, Antimicrobial Activity, B. anthracis spore, Biofilm.

IBE Abstract # 454

The Effects of the Electrical Double Layer on Giant Ionic Currents through Single Walled Carbon Nanotubes

Nanomaterials and Nanosystems

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Abstract

Electrofluidic transport through single walled carbon nanotubes (SWCNT) exhibit enhanced electrophoresis and electroosmosis, leading to total currents that are much larger than expected based on the conductivity of the bulk solution. To elucidate the underlying mechanism, a computational model of electrofluidic flow through a SWCNT was developed using the COMSOL Multiphysics by considering the complete Gouy-Chapman-Stern electrical double layer. The thickness of the compact layer was allowed to vary freely, resulting in a compact layer that increases with bulk solution concentration. The model was validated with internal consistency checks, external references, and empirical relationships. Results show that the observed increase in total conductance is due to enhancement of both electroosmosis and electrophoresis. Electroosmosis is enhanced by the perfect slip plane of the SWCNT and a net charge on the fluid due to the differential work function between the surrounding material and SWCNT. Electrophoresis is enhanced by an increase in the effective ion density within the SWCNT driven by the accumulation of charged species and the restriction of the SWCNT internal volume by a finite compact layer. Variation in the compact layer changed the dielectric conditions inside the SWCNT, resulting in the changes observed in the conducting solution. Aside from complying with conditions well-known to electrochemical theory, the model and results presented here agree well with experimental observations.

IBE Abstract # 522

Ag-TiO2-CNT Nanoparticles for Environmental Remediation: Synthesis, Characterization and Application

Nanomaterials and Nanosystems

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Abstract

Carbon nanotubes (CNTs)-modified with silver titania (Ag-TiO2) nanoparticles were synthesized via a photochemical reduction method. Field emission-scanning electron microscopy (FE-SEM), scanning transmission electron microscopy (STEM), energy dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), UV-Vis diffuse reflectance spectroscopy (DRS), and Raman spectroscopy were used to characterize the Ag-TiO2-CNT nanoparticles.It was confirmed that the surface of CNTs was uniformly decorated in a "aciniform-like" fashion with Ag-TiO2 particles of an average particle size of about 20-30 nm.Photocatalytic activity of Ag-TiO2-CNT nanoparticles was evaluated by studiesof methylene blue (MB) dye removal under artificial light irradiation by examination of adsorption and photo-oxidative effects. We observed an enhanced effect of oxidationof MB due to adsorption and photo-electrochemical properties of the Ag-TiO2-CNT nanoparticles due to spatial and electronic configurations. The ratio of CNT/TiO2 was an important variable in terms of balancing adsorption and degradation to get the best efficiency. Ag-TiO2-CNT nanoparticles with 1.0 % CNT/TiO2 by weight degraded MB with the best efficiency. These results show that Ag-TiO2-CNT nanoparticles may have potential applications for mitigation of organic pollutants in aqueous systems.

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Seeded Nanodiamond Surfaces for Bacterial Biosensing

Nanomaterials and Nanosystems

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Abstract

Summary: Tethering of biomolecules to transducing surfaces is inevitable in the process of creating biosensors. This requires the tethered biomolecules to stay active while tethered plus the surface chemistry to stay intact when exposed to working solutions. CVD grown nanocrystalline diamond films and its UV-alkene surface chemistry have been reported for hydrolytic stability and sustaining stability of tethered proteins better compared to silicon, gold, silica or glassy-carbon electrodes. However the production of CVD nanocrystalline diamond films is an expensive and energy consuming process. This talk reports our findings with an alternative approach of using detonation nanodiamonds to cover the surface of a gold electrode and functionalizing it using the UV-alkene chemistry for bacterial biosensing. Detonation nanodiamond can be considered as useful product out of environmentally friendly process for disposing old munitions. We studied the coverage obtained by the seeding process as a function of solution concentration and seeding time. The UV-alkene route of functionalizing antibodies on as seeded nanodiamond surfaces and hydrogenated nanodiamond-seeded surfaces was tested. In the end, we performed a set of experiments to correlate the effect of surface coverage of nano diamonds to the number of bacteria captured at several bacterial concentrations (10^5 - 10^7 cfu/ml).

IBE Abstract # 402

Engineering Whispering Gallery Mode Optical Biosensors for Environmental Monitoring

Sensors & Biosensors

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Abstract

Label-free biosensors that combine high sensitivity and high specificity characteristics have shown tremendous potential for applications in medical diagnostics, and have more recently been extended to the food safety and environmental monitoring arenas. Label-free optical biosensors, in particular, have become the workhorse for the biodetection and biodiagnostics industries due to their ease of use, non-destructive detection, and small sample size requirement. A unique type of label-free, optical biosensor, based on Whispering Gallery Mode microcavities, has tremendous potential to revolutionize biodetection due to its extreme sensitivity. The primary limitation of these biosensors, however, is that they require the addition of biorecognition elements to specifically target a biological species of interest. Therefore, the ability to selectively functionalize the microcavity for a specific target molecule, without degrading device performance, is extremely important, and represents the next step in translating these devices from laboratory to field environments. Here, we demonstrate a variety of straightforward bioconjugation strategies that not only impart specificity to optical microcavities, but also allow for biosensor recycling and the creation of multi-use platforms for complex environments. The resulting surface chemistry is illustrated with XPS, SEM, and fluorescence and optical microscopy. The device sensitivity is determined via quantitative microcavity analysis. The ability to minimize non-specific adsorption and target unique molecules in complex environments is demonstrated via ellipsometry and in situ device testing. The resulting devices can be recycled several times without loss of sensitivity, and can perform targeted detection in multi-component environments. By combining these high sensitivity biosensors with appropriate biochemistries, the resulting platforms can be applied not just to laboratory-scale

medical testing, but extended to address broader issues in environmental monitoring, such as water-borne pathogens.

IBE Abstract # 409

Micro and nanostructured lipid membranes for sensing and cell manipulation

Sensors & Biosensors

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Abstract

Cell membranes are vital components of cells in or at which a large fraction of cellular processes occur. Due to their complexity there is a drive to find reductionist model systems with which to address both fundamental biological questions regarding the organization and function of membrane constituents, the role of membrane organization and mobility in cell adhesion and interactions, and to implement them in industrial processes such as sensing and drug discovery. For all these application areas it is especially important to create membrane scaffolds that allow incorporation of functional membrane proteins in particular geometries and readout formats [1, 2].We have developed several platforms for studying the self-assembly of solid supported lipid bilayers from liposomes. Self-assembled lipid membranes, typically planar with macroscopic extension, allow a high degree of biomimicry and retention of functionality of delicate membrane components such as membrane proteins. However, for advanced sensor applications functional membrane assembly has to be combined with sensor structures. Our experience with controlling the assembly of membranes on sensor nanostructures will be presented with focus on membranes spanning nanopores. Nanopore-spanning membranes combine the robustness of supported lipid membranes with the additional space for large membrane proteins and electrochemical access to both sides of the membrane afforded by free-spanning membranes [1]. We also demonstrate the proof-of-concept of a novel nanoplasmonic sensor platform, which combines nanostructures, molecular patterning, directional and size-discriminating sensing especially suitable for membrane sensing.Important for membrane applications to biosensors and to study a multitude of interactions is to be able to pattern membranes on a substrate and to control the coupling of membrane protein binding motifs to supported membranes at controlled surface densities without reducing their functionality. We will present our work on patterning membranes both by lithographic methods on 2-D and 3-D substrates and by spotting of

membranes onto wetted substrates. We also present new strategies for coupling mobile ligands to patterned supported membranes under physiological conditions using biorecognition or enzymatic coupling.

1. Reimhult, E. and K. Kumar, Trends in Biotechnology, 2008. 26(2): p. 82-89.2. Reimhult, E., et al., Biotechnology and Genetic Engineering Reviews, Vol 272010. p. 185-216.

IBE Abstract # 411

Oxygen Uptake in Brassica Napus (Canola) at or near Swathing under Non-Lethal Stress

Sensors & Biosensors

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Abstract

During normal seed development, embryos develop from green photosynthetic cells to mature, desiccated and dormant forms which are devoid of chlorophyll. In various plants, including Brassica napus, developing embryos are green in color. During the maturation process, chlorophyll a/b ratio changes from 1.9:1 during degradation of chlorophyll; causing the color of the seed to changes from green to yellow. Normal embryos 'de-green' due to production of fatty acids, which prevents photo-oxidation of stored oil. Various environmental factors and agronomic practices alter the ability of a seed to rid itself of 'green color'. Frost and extreme hot/dry weather (at or near swathing) are the most common factors responsible for green seed formation. The green color is expensive to remove from the resulting oil through processing steps and significantly reduces oil yield. Canola (produced from B. napus seeds) is a major crop in Canada, providing 18% of farm revenues. Early frost at the seed pre-desiccation stage frequently provokes green seed formation in canola. There is an annual loss of $100M in farm revenue due to green seed incidence in North America. The biochemical process that leads to green seed formation due to non-lethal freezing has yet to be fully understood. The plant hormone abscisic acid (ABA) has been suggested to be involved in the control of seed de-greening because of its rapid decline in developing seeds following a freezing episode. It is also known that the normal seed development is under slightly hypoxic conditions. It is not clear how non-lethal freezing alters the microenvironment within B. napus seedpods. Therefore, this objective aims to determine the role of the hypoxic micro-environment on seedpod green seed formation in Canola. Understanding how oxygen interacts in controlling seed maturation within the unique microenvironment of the developing seed will provide new strategies to address the green seed problem in canola.

IBE Abstract # 414

Self-referencing Ca2+ sensors and differential imaging to study gravity response and physiology in Ceratopteris richardii

Sensors & Biosensors

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Abstract

The force of gravity has been constant throughout evolutionary history on Earth. However, the role gravity plays in developmental biology is poorly understood. Elucidating the gravity sensing mechanism used by plants to direct growth is one of the fundamental challenges in plant science. Previous gravitropic studies using an evolutionarily simple single-cell model system, spores of the fern Ceratopteris richardii, showed a tight correlation between cellular Ca2+ asymmetry and gravity-directed root outgrowth, a kind of gravitropism. The outgrowth follows gravitaxic migration of the nucleus directed by a transcellular Ca2+ gradient. Previous gravitropic studies using an evolutionarily simple single-cell model system, spores of the fern Ceratopteris richardii, showed a tight correlation between cellular Ca2+ asymmetry and gravity-directed root outgrowth, a kind of gravitropism. The outgrowth follows migration of the nucleus directed by a transcellular Ca2+ gradient. Understanding gravitropism will have profound impacts on agricultural and environmental engineering/sciences, which are intimately tied to knowledge of fundamental plant physiology. Expanded knowledge of gravitropism will contribute to protection of future food crops in stress conditions, and environmental cleanup via phytoremediation. Self-referencing (SR) microsensors are a unique and essential non-invasive tool for investigating the role of Ca2+ in gravity responses at the single cell level. SR sensors oscillate between two loci in order to measure transport of Ca2+ in a specific vector. Differential concentration (ΔC) measurement over a constant excursion distance (ΔX) provides direct measurement of flux based on Fick's first law of diffusion (J=D ΔC/ΔX). SR provides noise filtering capability that is not possible using other measurement technologies used to study transmembrane transport. In conjunction with self-referencing Ca2+ microsensors, a new differential imaging technique developed in the lab will be used to observe rhizoid outgrowth during SR measurement. Images are captured at a rate of 1 kHz, and pixelated images are correlated against a

reference image taken prior to germination. Differential images filter noise not associated with biologically distinct changes in morphology, which in turn increases resolution without the use of fluorescent dyes. By combining these non-invasive techniques, it will be possible to correlate Ca2+ movement with germination.

IBE Abstract # 420

Enzyme-based Biosensors for Studying Methanogenic Biofilm Physiology

Sensors & Biosensors

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Abstract

Waste streams from human activity provide a significant amount of potential energy that can be captured and converted to consumable fuel and reusable water. Human waste streams are commonly managed using anaerobic digestion, but the opportunity to extract energy from this complex process has not been widely adopted in municipal systems due to a lack of reliability and stability. Advanced research tools such as biosensors are required to improve understanding of the underlying biological processes driving the organisms involved in anaerobic digestion.The field of biosensors has recently seen increases in sensitivity, selectivity due to inclusion of catalytic nanomaterials; facilitating development of real-time and non-invasive designs. Microbiosensors have been effectively utilized to measure intracellular and extracellular analytes; such as molecules relevant to metabolism and cell signaling. There is a gap in the knowledge regarding biosensors that are specifically designed to investigate the physiological flux of methanogenic biofilm. The goal of this research is to expand scientific understanding of methanogenic biofilm and other microorganisms integral to biofuel generation by developing biosensors fabricated using nanomaterial based enzyme biosensors. In this research, an alcohol oxidase (AOx)-based methanol biosensor was fabricated and performance was characterized (sensitivity, selectivity, response time and operating range). AOx was immobilized together with platinum and carbon nanomaterials on a platinum/iridium electrode. Electro-catalytic nanomaterials such as carbon nanotubes and graphene enhance electron transport (i.e. increase sensitivity), while the catalytic reactions between methanol and alcohol-oxidase provide highly selective and reliable measurements. Cellulose-based biocompatible nano-materials such as microfibrous cellulose (MFC), cellulose nanocrystals (CNC) and saponite were used to immobilize enzymes through encapsulation and to improve performance and durability. The nano-

material-mediated AOx micro biosensor will be used to study methanogenic biofilm physiology and response to shock. Temperature, pH and substrate concentration will be adjusted to simulate common anaerobic digestion scenarios while alcohol flux is continuously measured. This research will improve understanding of change(s) in methane generation for methanogenic biofilm under shock and stress conditions. This fundamental research will improve the design and operation of anaerobic digesters, possibly advancing new discoveries in reactor engineering and process control.

IBE Abstract # 425

Complex supported lipid bilayers with high cholesterol content formed by helical peptide-induced vesicle fusion

Sensors & Biosensors

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Abstract

The objective of this work is to establish helical (AH) peptide-induced vesicle fusion as a reliable and facile technique to form supported lipid bilayers (SLBs) containing a high cholesterol content and multiple lipid types. Vesicles consisting of POPC : POPE : POPS : sphingomyelin : cholesterol (9.35 : 19.25 : 8.25 : 18.15 : 45.00) were used to form a SLB that models the native composition of the human immunodeficiency virus-1 (HIV-1) lipid envelope. In the absence of AH peptides, these biomimetic vesicles failed to form a complete SLB. Methods: We verified and characterized AH peptide-induced vesicle fusion by quartz crystal microbalance with dissipation monitoring (QCM-D), neutron reflectivity, and high-resolution scanning-probe imaging. Our results showed that AH peptide-induced vesicle fusion is a reliable method to engineer supported lipid bilayers (SLBs) containing complex membrane compositions including a high concentration of cholesterol and membrane embedded peptides. Successful SLB formation entailed a QCM-D characteristic frequency shift of -35.4 ± 2.0 Hz and a change in dissipation energy of 1.91 ± 0.52 x 10-6. Neutron reflectivity measurements determined the SLB thickness to be 49.9 +1.9-1.5 …, and showed the SLB to be 100 +0.0-0.1% complete and void of residual AH peptide after washing. Atomic force microscopy imaging confirmed complete SLB formation and revealed three distinct membrane domains with no visible defects.Our research is significant in that it provides a biologically relevant system to screen protein-membrane interactions with a broad range of diagnostic tools. Given the success reported here and by Cho and coworkers in using AH peptide-induced vesicle fusion, there is potential for this technique to form SLBs under a range of conditions and surfaces that are generally unfavorable for spontaneous vesicle fusion.

IBE Abstract # 445

Cell-based Sensing: from 2D to 3D Cell Culture

Sensors & Biosensors

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Abstract

Cell-based biosensors use living cells or tissues as sensing element to monitor physiological and functional changes induced by external stimuli. They have become an important pillar of drug discovery process, to provide a simple, fast and cost-effective tool to avoid large-scale and cost-intensive animal testing. The sensing element---cultured cells, is the most critical part of a cell-based biosensor. To date, almost all cell-based biosensors use traditional 2 dimensional (2D) monolayer cells cultured on flat and rigid substrate as the sensing element. Although the time-honored 2D cell culture has proven to be a valuable method for cell-based studies, its limitations have been increasingly recognized. In *in vivo* environment, almost all cells are surrounded by other cells and extracellular matrix (ECM) in a 3D fashion. As a result, 2D cell culture tests sometimes give unsatisfactorily misleading and non-predictive data for *in vivo* responses.

Recent studies have shown that 3D cell culture provides a more physiologically relevant environment for cells and allows the study of cellular responses in a setting that resembles *in vivo* environments. The 3D structure not only influences the spatial organization of the cell surface receptors engaged in interactions with surrounding cells, but also induce the physical constraint to cells. These spatial and physical aspects in 3D affect the signal transduction from the outside to the inside of cells, and ultimately influence on gene expression and cellular behaviors. Compared to 2D cell culture, 3D culture replicates more accurately the actual microenvironment where cells reside in tissue and therefore the behavior of cells in 3D culture reflects closely the *in vivo* responses.

This study focused on the adoption of 3D cell cultures to cell-based biosensors, aiming to provide more *in vivo*-like experimental results for drug discovery. We developed 3D cell culture systems for different cancer cell lines. Cell proliferation and cellular responses to different anti-cancer drugs in 3D cell culture were determined and compared with those in 2D cell culture.

IBE Abstract # 462

Non-Destructive Optical Oxygen Sensing Using PtTFPP (Platinum Tetrakis(Pentafluorophenyl) Porphyrin)On Long Term Shelf-Life Study For Food Packaging

Sensors & Biosensors

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Abstract

Modified Atmosphere Packaged (MAP) food applies a protective gas mixture, which normally contains either carbon dioxide (CO2) or nitrogen (N2), in order to extend the shelf life of food. Conventional MAP analysis of package temper study involves destructive sampling of packages to detect current oxygen concentration inside. The concept of non-destructive optical oxygen sensing is highly desirable for long term shelf-life study of MAP food packaging for quality control reasons.

The objective of this work was to develop optical oxygen sensor with PtTFPP (platinum tetrakis(pentafluorophenyl) porphyrin) and monitor oxygen concentration variation over time. The polymer matrix provides mechanical stability to the immobilized membrane and entraps the fluorescent dye (PtTFPP) molecules. This optical interaction between oxygen and fluorescent dye dynamically changes the fluorescence lifetime of the dye. The oxygen sensing is achieved by detecting degree of changed fluorescent lifetimes. Dye is encapsulated in polystyrene compatible to current commercial packaging.

Food packaging samples with different gas mixture ratio were tested in our work to provide wide range of oxygen detection over time with high precision and accuracy. It monitored increase of oxygen concentration inside samples over 12 month which proved durability of our dye sensor.

The sensor performance compared well with commercially available destructive oxygen concentration measurement (MOCON). The results of preliminary in-packaging trials are presented and future plan of fabricating on-packaging oxygen indicator is discussed.

Supported by Nestle-Gerber.
Keywords: sensor, optical, oxygen, food packaging

IBE Abstract # 470

Comparative studies on nanomaterial platforms for biosensors to monitor meat quality

Sensors & Biosensors

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Abstract

The ability to rapidly screen for toxins is a vital tool for maintaining a safe supply of high quality foodstuffs of animal origin (e.g., fish, pork). Handheld biosensors which target byproducts formed during the degradation of meat (e.g. xanthine/hypoxanthine, inosine, adenosine triphosphate) have many advantages over conventional laboratory analysis. Codeposition of nanomaterials improves the sensitivity and response time of biosensors. This study aims to compare the electrochemical behavior of three nanomaterial platforms for biosensors: amorphous platinum black (nPt), single layer graphene oxide (GO), and a composite of both nanoplatinum and graphene oxide (nPt_GO_nPt). These platforms were assembled onPt/Ir working electrodes and tested against Ag/AgCl reference electrodes. A performance characterization (sensitivity, selectivity, response time, range, hysteresis) was conducted using cyclic voltammetry in potassium ferrocyanide (K3Fe(CN)6) and DC-potential amperometry in phosphate buffer solution (PBS). Morphological aspects of the nanomaterial were evaluated through optical profiles and electron microscopy. Comparative results on electroactive surface area, surface roughness, sensitivity and response time show a significant enhancement of electrical properties of the nPt_GO_nPt functionalized electrode relative to the other nanomaterial platforms. The nanocomposite platform will be useful for development of electrochemical biosensors in real time monitoring of biomarkers related to meat spoilage.

Key words: nanomaterial platforms, graphene oxide, platinum black, amperometric biosensors, meat spoilage.

IBE Abstract # 472

DEP Manipulation of Polystyrene Beads

Sensors & Biosensors

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Abstract

Introduction: Dielectrophoresis (DEP) can be used to manipulate particles for lab-on-a-chip applications. This technique involves the movement of particles with no net charge (dielectric) when exposed to a non-uniform electric field. This differs from electrophoresis in that electrophoresis involves the movement of charged and non-charged particles. The dielectric particles used in these studies are polystyrene beads ranging from 2µm to 10µm in diameter.

This study was to investigate the operation and efficacy of relatively low voltage dielectrophoresis for the purpose of micron-scale particle arrangement and manipulation. With this goal in mind, we also performed initial quantification of the forces involved in this process.

Methods: The main setup used for this work consists of interdigitated electrodes, a thin plastic sheet (12µm thick), and polystyrene beads of various sizes suspended in deionized water. The interdigitated electrodes were etched out of a thin titanium/gold film deposited on a glass slide using conventional photolithographic techniques. Several electrode shapes including straight, circular and square parallel lines were used. The thin plastic sheet was placed on top of the electrodes as an insulating layer. During experiments, drops of DI water containing polystyrene beads were placed on the plastic sheet while the electrodes were biased under an AC potential field at various voltages and frequencies (0-32Vpp and 0-20MHz). The induced movement and alignment of the beads by the applied the electrical field were monitored using an optical microscope.

Results: Under an AC electrical field, “pearl chains” of polystyrene beads formed readily between the interdigitated electrodes within certain frequency bands once sufficient voltage was applied (~16Vpp). Other particle arrangements were also repeatedly induced at frequencies outside these bands. It was also

found that at a higher frequency, a lower voltage was required to lift the beads off of the plastic film. The conductivity of the medium has a significant influence over the response of particles suspended in the medium to applied electric fields.

Conclusions: It has been demonstrated that it is possible to dielectrophoretically position polystyrene beads using voltages as low as 16Vpp with a simple setup. This voltage is significantly lower than that used in other similar studies.[1] The use of the plastic sheet also allows the electrode to be reused, thus further reducing cost. Future work will be done to achieve a more complete characterization of the vertical (lifting) component of the force due to the induced electric field. Our experimental results agree well with our computational modeling results as well as other theoretical results.[2] Moreover, we are now working on using the dielectrophoresis to manipulate cells in suspension for the purpose of patterning cells on devices for tissue engineering or other possible applications.

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IBE Abstract # 474

Directed functionalization strategies for high-resolution optical fiber based biosensors

Sensors & Biosensors

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Abstract

Physiological sensing is inherently a multi-scale problem that can involve measurements of multiple analytes from single cells or complex cell networks and usually spans a broad range of spatial and temporal scales. An ideal sensing paradigm typically needs to have high sensitivity and selectivity, good signal to noise ratio, drift compensation, fast response (for high temporal resolution) and an easily scalable sensing area (for tunable spatial resolution). In recent years, novel nanomaterials and processing techniques have enabled a new generation of sensing elements that have significantly enhanced sensor sensitivity and temporal resolution. However the design of better functionalization schemes for reliable coupling with the recognition element lags behind. The goal of this project is to develop site-directed functionalization strategies for high spatial resolution optical fiber based biosensors.Previous work at the Physiological Sensing Facility has shown that the use of frequency domain fluorescence lifetime based optrode schemes in conjunction with optimal selection of titanium oxide microparticles allows enhanced oxygen detection in both direct and self-referencing mode. However, these schemes relied on a simple dip-coating protocol for dye incorporation at the end of a laser pulled multimode optical fiber tip, limiting the minimum attainable tip size to approximately 20 um. We build on this work to develop micron to sub-micron self-referencing optrodes using photo-initiated polymerization and plasmon enhanced fluorescence. Proper selection of monomer, photoinitiator and excitation source enables controlled incorporation of different classes of analyte-sensitive fluorescent transducers onto the tips for high resolution sensing. Growing tips can also be doped with plasmonic materials to tune optical properties of the incorporated transducer molecules. An oxygen sensitive fluorophore and a glutamate sensitive FRET

protein are used as model systems to demonstrate the flexibility of this functionalization scheme. Polymerization parameters and dopant levels are optimized for fluorescence lifetime based sensing in the case of the oxygen optrode and for ratiometric, fluorescence intensity mode measurements in the case of the glutamate optrode.

IBE Abstract # 479

Lab-on-a-Chip Technology utilizing All-solid-state ion-selective electrode (ASISE) Approaches for Microfabricated Biological Sensors

Sensors & Biosensors

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Abstract

Physiological sensing conducted in a liquid environment requires electrodes with long lifetime. The development of a robust microfabricated all-solid-state ion-selective electrode (ASISE)–based biochip in a lab-on-a-chip (LOC) platform is described. To compare ASISEs lifetime, which is driven by the transducer layer based of conductive polymer poly(3,4-ethylenedioxythiophene) (PEDOT), electrochemical measurements were performed in a custom-made microfluidic flow-cell chamber. The results of potentiometric measurement of cationic analytes demonstrate the electrodes to have a near-Nernstian slope profile even after they are stored for almost three months in liquid medium. The electrodes also achieved H2O2 amperometric sensitivity (1.25 and 3.32 μAmM-1cm-2 for PEDOT: poly(styrenesulfonate) (PSS) and PEDOT:calcium sulfate (CaSO4) respectively) and lower detection limit (2.21 μM, 8.4 μM, 3.44 μM, for H+, NH4+, Ca2+ respectively) comparable to that of wire-type electrodes. Furthermore, the lifetime is dependent on the electrodeposition method of the conductive polymer, and the transducer layer must be modified to fit the analyte types. These results indicate that extended lifetime of microfabricated ASISEs in a multiplex format can be realized by optimizing the microfabricated electrode surface functionalization. These robust ASISEs were used in several sensing systems. This include applications in cells' physiological sensing; they are calcium-ion monitoring of mammalian cells (CHO-K1) and plant single cells (fern spore Ceratopteris richardii), and simultaneous monitoring of calcium-ion and pH in green algae (Chlorella vulgaris). ASISEs were also used to provide water quality monitoring, by measuring H+, NH4+, Ca2+, and Mg2+ ions, which are the key ions for pH, water hardness, and disinfectant. The applications in these sensing systems will be demonstrated, and the measurement results confirm the robustness of the ASISEs to be utilized for various applications. This

technology has the potential to be modified for other biological and medical applications both on Earth and in space systems.

IBE Abstract # 480

Chinese Hamster Ovary Cell Culture on All-Solid-State Ion-Selective Electrodes

Sensors & Biosensors

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Abstract

A microfabricated lab-on-a-chip platform allows for in situ multiplex sensing of physiological events. The development of a biochip for multiplex ion sensing, named the multi-analyte biochip (MAB), for in vitro cellular measurements is presented. The MAB consists of multiple all-solid-state ion-selective electrodes (ASSISE) intended for cellular measurements in complex biological cell-culture media. Multiple electrodes allow for multiple measurement sites, reducing the dispersion in data that is inherent to measurement of biological activity. An ASSISE consists of a transducing conductive polymer layer and an ion-selective membrane (ISM). The direct use of ASSISEs for cellular measurement is not straightforward; in particular, electrode lifetime and biocompatibility are important factors. The ISM consists of polymers and ionophores that could be detrimental to cell viability. Furthermore, these media contain many interfering salts and fouling compounds that could affect measurements. We have performed experiments to test cell growth and cell morphology on the MAB. Chinese hamster ovary (CHO-K1) cells were cultured on calcium ion-selective electrodes. No aberrant effect on cell growth or morphology was seen in confocal microscopy. Methylthiazol Tetrazolium (MTT) assay indicated that the mitochondrial activity and cell viability was not affected by ISM. The cells were found to retain their growth curve, and the morphology changed pattern according to the growth cycle, similar to cells cultured on short peptides. After a two-week experiment, we confirmed that a calcium ion-selective electrode could still function with consistent potentiometric and amperometric sensitivity. Calcium-ion activity and signaling of mammalian cell arrays has been of recent interest in cell electro-physiology and neurophysiology. From this biocompatibility test results, robust ASSISE will be employed to perform

continuous monitoring of calcium-ion activity and signaling of mammalian cell arrays. CHO-K1 cells are grown directly on the MAB surface, and calcium current will be measured for more than 36 hours. This is the first demonstration of multiplex ASSISEs use in monitoring physiological activity of mammalian cells. The results will have impact on cell-electrophysiology research, where the method is not only non-invasive but also biocompatible for other cell array types.

IBE Abstract # 490

Extremely Fast Nucleic Acid Amplification by Droplet Manipulation for Point-of-Care Diagnosis of Blood Infection

Sensors & Biosensors

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Abstract

Introduction: Cell culture has typically been used to help diagnose clinical infection within 2-3 days. It is subject to error due to competitive growth, biased selection by culture conditions and neglect for species which lack colony forming capacity in vitro. Real-time Polymerase Chain Reaction (PCR) is an attractive alternative technique for clinical diagnosis of infection in clinical laboratories. In the past, complicated sample preparation, the need for sophisticated laboratory equipment, and thermocycling time have prohibited the bedside and intraoperative use of PCR, leaving doctors reliant solely on their clinical judgment of signs of infection and empiric antibiotics until laboratory results are received. In order to address these barriers to clinical PCR and to fill the temporal gap between clinical decision making and receipt of laboratory results, we have designed a droplet bioreactor for in situ bacterial culture, sample preparation, nucleic acid amplification and real-time quantitative detection of a panel of clinically relevant bacteria.Materials and Methods: Our device has three thermally isolated baths of heated silicone oil oriented in a circular fashion. This circular chamber design allows for a syringe to be rotated between chambers using a single stepper motor. A separate stepper motor controls the vertical movement of the syringe in and out of the oil. The syringe plunger is controlled by a linear actuator, making it possible to reliably and repeatedly dispense a 10 microliter droplet from the syringe needle. In order to utilize convective heat transfer, the droplet is submersed and moved in the silicone oil bath at 2 cm/s while hanging from the syringe needle tip. The temperature and motors are controlled by an Arduino microprocessor.Universal PCR primers were designed to amplify the V6 hypervariable region of the 16s rRNA gene from all species of bacteria. Sequence specific fluorogenic hybridization probes were designed for specific detection of a panel of clinically relevant bacteria. The sequences of these probes where blasted against the Ribosomal Database Project to determine their sequence specificity. These

probes provide a real-time fluorescent signal which can be linearly correlated to the initial target concentration in the sample.Results and Discussion: The V6 hypervariable region of the 16s rRNA gene has been amplified using conventional PCR thermocycling using Escherichia coli, Salmonella, and Staphylococcus aureus. The blast results for the Staphylococcus aureus hybridization probe revealed a greater than 98% specificity. Three different temperatures were successfully maintained within 2 degrees Celsius of set point temperatures in each chamber. The device has been tested using a droplet containing E. coli DNA, primers for the V6 hypervariable region of the 16s rRNA gene, and PCR reaction reagents. A 10 microliter droplet was maintained on the syringe needle and collected after 30 cycles of PCR thermocycling. Conclusions: Our device provides a platform for real-time diagnosis of infection providing the opportunity to eliminate the necessity for doctors to rely on clinical signs and judgment when treating patients with infection. The design of this system also affords the opportunity to increase throughput by using arrays of syringes and larger silicone oil baths. The hybridization probes also lend themselves well to a multiplexed system in that they can be designed to fluoresce at different, resolvable wavelengths. Our method of convectively heating a bare droplet in silicone oil reduces the time required for heat transfer significantly making this concept ideal for the time sensitive arena of the clinic.

IBE Abstract # 492

Paper Microfluidics Detection of Salmonella Using a Smart Phone

Sensors & Biosensors

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Abstract

Paper microfluidics has gained great popularity in recent years, potentially as a low-cost, field deployable assay device. In this work, we demonstrated, for the first time, the immunoagglutination assay in a paper microfluidics and its Mie scatter detection using the smart phone's flash and digital camera as an optical sensing platform. The paper microfluidics was fabricated using cellulose chromatography paper (Whatman) and SU-8 photoresist. Anti-Salmonella were conjugated to 920 nm, highly carboxylated polystyrene beads by covalent bonding as described previously. This bead suspension was applied to the detection area of paper microfluidics and fully dried. The final paper microfluidics would have multiple channels, each with different antibody-conjugated beads pre-loaded in it, detecting multiple pathogens simultaneously. The paper microfluidics is dipped into the target Salmonella solutions (together with a blank = phosphate buffered saline), which travels through the channel by capillary action. The target molecule (Salmonella) in the solution causes immunoagglutination of antibody-conjugated beads within paper fibers, thus increasing the effective diameter and morphology of the beads, which is detected through light scatter intensities. This is a label-free, non-spectrophotometric detection, which improves the detection limit down to 10 CFU/mL bacteria or 10 pg/mL antigens. The tradeoff is the difficulty in differentiating the true light scatter caused by the bead immnoagglutination from the reflection or non-specific scatter by the paper fiber. Therefore, a benchtop apparatus was fabricated using fiber optic cables, blue (lambda = 475 nm) LED light source and a miniature spectrometer, and the angles of light irradiation and detection were varied using rotational positioning stages. Forward scatter (i.e. light passes through the paper) from 0 degree to 80 degrees and back scatter (i.e. light reflects from the paper) from 100 degrees - 160 degrees were tested. Due to the non-transparent nature of the paper, back scatter showed stronger signal, and the optimum angle was obtained at 150 degrees. Using this setup and the optimized angle, the

light scatter intensities were collected varying the target (Salmonella) concentrations. All data were normalized to that of a blank (PBS), and a standard curve was constructed. The curve shows initial increase, followed by a dip, which is purely an optical phenomenon of Mie scatter as demonstrated previously by Mie scatter simulations, then by a continued increase until the antigen saturation occurs at 10^5 CFU/mL. This dip will become less pronounced through reducing the size of channels (thus limiting the growth of beads only up to doublets or triplets). The whole experiment was repeated, this time using two cell phones, one as a light source (white flash) perpendicular to the paper and the other as a detector (digital camera) at 150 degrees. The resulting standard curve shows exactly identical trend. All assays showed the detection limit of 10^2 CFU/mL. The proposed system demonstrates a strong potential to be used in field situations at extremely low cost.

IBE Abstract # 519

Nano-Dielectrophoresis Chip Integrated with Raman Spectroscopic self-referencing Detection of Foodborne Pathogens

Sensors & Biosensors

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Abstract

Nanoenabled dielectrophoresis (nanoDEP) chip was fabricated by utilizing a nanoelectrode array made of vertically aligned carbon nanofibers (VACNFs) versus a macroscopic indium tin oxide electrode in a points-and-lid configuration integrated in a microfluidic channel. It was demonstrated that the nanoDEP-chip can effectively capture pathogenic bacterial cells as well as viral particles with high efficiency (>70% for bacteria, >60% for virus), at high flow rate (100 mL/hr). The captured targets were subsequently interrogated at their capturing sites by self-referencing dual-recognition Raman spectroscopic fingerprinting. In this approach, Raman nanoprobes were made by functionalizing Raman-labelled nanoparticles with antibodies that recognizing specific surface markers of a pathogen target (e.g., E. coli O157:H7). At least two different probes were made for each target pathogen, i.e., they bind to different surface epitopes of the same pathogen. Specific molecular recognition signals (e.g., signals indicating antibodies binding to target cells) were then evaluated in parallel with inherent target spectroscopic signatures by surface-enhanced Raman spectrosensing (SERS). A positive I.D. of the target was recorded only when signals from different probes (at least two different types) and the target itself were obtained simultaneously, indicating binding of probes to their specific targets has occurred. By introducing the self-referencing cross-validation between the two independent I.D. signaling channels, the accuracy of the detection for different pathogen target was greatly improved. Alongside with the nanoDEP capturing, highly accurate detection of pathogenic targets at 1-10 cell sensitivity was achieved. The nanoDEP SERS sensor potentially can become a powerful tool for rapid detection of pathogens that offers supreme sensitivity and specificity.

IBE Abstract # 553

Graphene Bio-Nanosensing

Sensors & Biosensors

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Abstract

The promise of designing a platform consisting of miniaturized biomimetic nanosensor for the highly sensitive, selective, and label-free detection of analytes can stimulate revolutionary scientific and technological opportunities in medical, environmental, defense and aerospace applications. Graphene is a single-atom-thick, two-dimensional carbon based material with remarkable electronic and sensing properties. Biological smart materials with integrated nano-transducer can provide a general platform for highly sensitive and selective nanosensors. Here, I will describe the selection of specific bioreceptors for recognition, the fabrication of graphene-based electrodes, the immobilization of bioreceptors on graphene, and the electrical characterization of the biofunctionalized graphene-electrodes as specific nanosensors for detection of target analytes.

IBE Abstract # 421

Development of a Biophysical Model of Translational Coupling

Synthetic Biology

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Abstract

Coordination of multiple enzyme expression is often required to maximize metabolic pathway flux. In bacterial polycistronic operons, translational coupling is a common mechanism for coordinating protein expression, including Trp operon in E.coli, ATP operon in E.coli and the operon that encodes type III secretion system in Salmonella. Translational coupling occurs when translation of an upstream gene increases the translation rate of a downstream gene through ribosome-assisted unfolding of the intergenic mRNA hairpins and ribosome re-initiating of the downstream mRNA translation. To quantify the relationship between upstream and downstream mRNA translation rate, we developed a biophysical model of translational coupling to predict downstream translation initiation rate and to engineer multi-cistronic operons where protein expression will have a target stoichiometry. Our biophysical model uses the upstream mRNA sequence, the thermodynamic free energy of the intergenic mRNA hairpins, and the intergenic distance between two coupled genes to calculate the translation initiation rate of the downstream mRNA. We experimentally validated the model by constructing bi-cistronic operons, expressing red and green fluorescent proteins, and employing flow cytometry to record the protein expression levels. Interestingly, we found that translational coupling reduces the gene expression stochasticity in single cell. The model validity in multi-cistronic coupling systems was also demonstrated by engineering three-color operons expressing cyan, red and green fluorescent proteins. Our findings highlight the potential of a genome-wide prediction of downstream translation rate using mRNA sequence in natural coupling cistrons, and a de novo designing of the intergenic mRNA sequence that generates the desired protein expression stoichiometry.

IBE Abstract # 458

Economic production of Polyhydroxyalkanoates in Escherichia coli

Synthetic Biology

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Abstract

Traditional plastics are derived from petroleum and are non-biodegradable. Currently, there is a push to reduce the dependence on petroleum derived products and a move towards biodegradable products. Polyhydroxyalkanoates (PHAs) are a group of biodegradable bioplastics that are produced by a wide variety of microorganisms, mainly as a storage intermediate for energy and carbon. PHAs have similar melting point to polypropylene and a Young's modulus close to polystyrene. There are two major issues restricting the large scale production and utilization of PHAs: 1) the downstream processing of bacterial cultures to extract the valuable PHA and 2) cheap carbon substrate. Secreting PHA from Escherichia coli could potentially reduce downstream processing costs as it will be easier to separate secreted PHA from the biomass. Secreting PHA utilizes the process of type I secretion with hylA signal peptides. Secretion should enable the recombinant bacteria to have increased production of PHAs, and to continue to produce PHA that does not need to be harvested using traditional toxic solvents methods. To help understand secretion of PHAs, the green fluorescent protein (GFP) can be tagged to the PHA polymerase enzyme encoded by phaC. phaC is part of a three gene cassette that includes phaA and phaB and are required for protein expression and ultimately PHA production. In addition to GFP, visualizing the secretion process with SEM and TEM can further enhance the understanding of the secretion phenomena. To address the issue of expensive carbon substrate, cheap alternatives can be used. The wet lipid extraction process (WLEP) uses algae grown on wastewater as a feedstock to produce biodiesel. One of the side streams of the WLEP is the aqueous phase which is rich in simple sugars such as glycerol. Growing the bioplastic producing bacteria on this substrate can potentially make PHA production economically feasible.

IBE Abstract # 461

Synthetic Biology and Bioinformatics for Predictable Control of Therapeutic Genes

Synthetic Biology

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Abstract

Contextual sensitivity is an engineering challenge that faces the progress of synthetic biology applications. Protein-DNA packaging of human (and generally, eukaryotic) chromosomes can influence synthetic system function in unexpected ways. We have developed a bioinformatics-driven platform for identifying and predicting sources of contextual variance of artificial regulation of endogenous genes. In this work, we focused on a synthetic transcription factor, called Pc-TF, which can be used to activate anti-cancer genes (1) and, potentially, tissue regeneration pathways. Pc-TF is designed to target the histone methylation mark H3K27me3, and activate nearby promoters of genes. H3K27me3 may appear at roughly 1000 different promoters in different cell types, we asked whether Pc-TF activity is context-dependent. We employed a variety of bioinformatics tools to predict how Pc-TF will regulate genes and, consequently, cell phenotype in three different human cell types. H3K27me3-associated promoters of genes were identified from ENCODE chromatin mapping data (2) in liver carcinoma (HepG2), erythroleukemia (K562), and neuroblastoma (SK-N-SH) using GALAXY (3), an open web-based platform. When we compared the predicted Pc-TF target gene lists, we found that a majority of target genes were unique to each cell line. This suggests that Pc-TF will induce a different change in phenotype depending upon the type of cell it is expressed in. To investigate this idea, we performed Gene Ontology Term enrichment analysis (using GOrilla, 4) on the predicted Pc-TF target gene lists. We found that the target biological processes, molecular functions, and cellular components were largely distinct ($p < 1.0E3$). This result suggests that Pc-TF will control cell phenotype in a context-specific manner. Currently, we are measuring changes in predicted target gene expression by comparing RT-PCR results of each cell type, before and after Pc-TF protein production. This work will lead to an efficient, effective, and broadly applicable procedure for predicting context-dependent function of synthetic transcription

factors. Our work will demonstrate how coupling bioinformatics with synthetic biology can eliminate the guesswork in developing molecular therapeutics.

IBE Abstract # 483

Development of Flavin-based Fluorescent Proteins for Biological Imaging

Synthetic Biology

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Abstract

In this work, we report on the development of flavin-binding photosensory proteins as new fluorescent reporters with potential applications in synthetic biology and biological engineering. Flavin-based fluorescent proteins (FbFPs) were recently reported as novel reporters that are characterized by small size and oxygen-independent maturation of fluorescence, which are key advantages over the widely used green fluorescent protein (GFP). However, FbFPs are at a nascent stage of development and a full understanding of the performance and properties of FbFPs as a practical set of biological probes is lacking. Therefore, we extensively characterized the existing set of FbFPs in vitro in terms of brightness, oligomeric state, maturation time, fraction of (fluorescent) holoprotein, pH tolerance, redox sensitivity, and thermal stability. Overall, FbFPs show important advantages as broad-spectrum biological reporters, including robust fluorescence over a broad pH and temperature range (pH 4-11, up to 60 C) and rapid maturation of fluorescence (< 3 min. vs. 30 min. in GFP). In addition, we validated FbFPs as stable fluorescent reporters in vivo by constructing a series of FbFP-based transcriptional constructs to probe promoter dynamics in Escherichia coli (see attached figure 1). Furthermore, we applied directed evolution and isolated two FbFP mutants that show a 2-fold enhancement in fluorescence emission over the parent protein (see attached figure 2). Finally, we are developing a molecular framework to computationally focus identification and engineering of FbFPs with novel properties. In particular, we are developing a â€œmolecular fingerprintâ€ for FbFPs by integrating structural, thermodynamic, and evolutionary data on amino acids comprising the flavin-binding pocket in homologous proteins (see attached figure 3). Based on the fingerprint, we predict a mutational phase space for improving fluorescence and thermal

stability in FbFPs through model-guided protein engineering. In this way, we adopt a comprehensive approach to understand and improve FbFP-based imaging technology. We anticipate that our work will enable the broad application of FbFPs and their bright mutants as reporters of gene expression, protein localization, and whole cell microbial sensors in conditions where GFP-based reporters fail to perform optimally (e.g., fast time-scale biological processes, extremophilic microbes, anaerobiosis).

IBE Abstract # 498

Estimation of gene network parameters from single-cell fluorescence trajectories

Synthetic Biology

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Abstract

Synthetic biology endeavors to drive design of novel genetic constructs through predictive models of the behavior of individual components. However, parameterization of these models is difficult because the networks are complex, the number of measurable quantities in vivo is low, and noise is high. Significant progress has been made by considering the relative performance of component variants, but stochastic models parameterized in absolute terms promise to improve our ability to predict the behavior of constructs in detail. Here we present our efforts to obtain such parameters. We have developed a novel instrument, GenoSIGHT, that integrates time-lapse fluorescent microscopy and microfluidics to collect single-cell trajectories of cells grown in a highly controlled, continuous-flow environment. We then leverage the information-rich data to estimate rates for a stochastic model of a fluorescent protein expressed from an inducible promoter. After careful parameterization, stochastic simulations of our model faithfully mimic the evolution of the distribution of fluorescence per cell over the course of each experiment. The datasets strongly constrain these rates to specific values, with the exception of the translation rate which has multiple solutions because it is obscured by the slow maturation dynamics of the fluorescent reporter. We verify our model by measuring the number of mRNA copies per cell via Fluorescence In Situ Hybridization (FISH), and show that our model accurately predicts the evolution of mRNA counts over time. Automated instruments like GenoSIGHT capable of collecting information-rich datasets, combined with advanced methods of parameterizing stochastic models from such datasets, herald a paradigm shift towards truly predictive engineering of genetic constructs.

IBE Abstract # 520

Temporal control of self-organized pattern formation without morphogen gradients in engineered bacteria

Synthetic Biology

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Abstract

Diverse mechanisms have been proposed to explain natural pattern formation processes, such as slime mold aggregation, feather branching, and tissue stratification. Regardless of the specific molecular interactions, these mechanisms assume the presence of morphogen gradients, which are either predefined or generated as part of the patterning process. However, using E. coli programmed by a simple synthetic gene circuit, we demonstrate here the generation of robust, self-organized ring patterns of gene expression in the absence of an apparent morphogen gradient. Interestingly, our modeling and experimental tests show that the temporal dynamics of the global morphogen concentration serve as a timing mechanism to trigger formation and maintenance of these ring patterns, which are readily tunable by experimentally controllable environmental factors. This mechanism represents a novel mode of pattern formation that has implications for understanding natural developmental processes.

IBE Abstract # 543

Engineering safeguard mechanism for microbial swarmbots

Synthetic Biology

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Abstract

Engineered cell-based sensors and actuators have great potential for diverse applications in medicine, bioremediation and energy. These applications, however, have been limited by the controllability, scalability and public safety concerns regarding unanticipated effects of engineered bacteria on human health and environment if they escape from controlled environment. To address this issue, we are developing a "microbial swarmbot" technology using soft photolithography to fabricate the hollow alginate-poly-L-lysine-alginate (APA) microcapsules for encapsulating engineered bacteria. Using APA microcapsules as our platform for microbial swarmbot technology, we would like to establish a safeguard mechanism for effectively killing escaped bacteria with a self-addiction gene circuit. We have employed MC4100 Escherichia coli strain engineered to constitutively express β-lactamase (BlaM) gene to examine the circuit behavior for establishing a safeguard mechanism. Since BlaM can only degrade β-lactam antibiotics extracellularly, cells can only survive with help from nearby cells producing and releasing BlaM. Our preliminary results demonstrated that the escaped bacteria cells from microcapsules would not survive outside the microcapsule environment because the local cell density outside the microcapsules is insufficient to produce enough BlaM to support survival. As such, our circuit and the corresponding encapsulation strategy have defined a novel approach to prevent unintended proliferation engineered bacteria for novel applications, particularly in autonomous synthesis and delivery of proteins.

IBE Abstract # 521

Constructing a Synthetic Gene Network to Model and Understand Signaling Interactions in Drosophila melanogaster

Synthetic Biology

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Abstract

In multicellular organisms, cellular signaling events are crucial for patterning tissues, as well as for maintaining healthy adult tissues, while improper signaling can lead to disease states, such as cancer. Therefore, cellular signaling process must be tightly regulated. A complex system of gene regulatory circuits controls this signaling process and acts to buffer this system against noise, thereby minimizing mistakes in gene expression and preventing patterning defects or disease states. Despite their importance to patterning and development, hypotheses regarding these gene regulatory circuits have been difficult to test experimentally due to their complexity and high connectivity. Therefore to better understand the fundamental processes involved, we created a synthetic gene network in the fruit fly Drosophila melanogaster. This network utilizes genes from yeast and E. coli, namely, gal4, gal80, and lacZ. We expressed gal4 in a graded fashion along the anterior-posterior axis of the embryo, mimicking the endogenous transcription factor, Bicoid. The gal4 activates expression of UAS-linked gal80 and lacZ. gal80 inhibits gal4 activation, creating a negative feedback loop in our system. These genes were chosen since they are not endogenous to Drosophila, so all interactions in this network will be fully understood. Our goal is to measure the variability in location of the lacZ domain both with and without the negative feedback loop. This synthetic system provides a direct experimental test of whether negative feedback loops in muticellular systems such as Drosophila, can provide robustness to noisy, diffusive systems. It is this robustness that is important for combating diseases and defects in development and maintenance of expression in the organism.

IBE Abstract # 463

Hydroxylated Flavones Reduce Alzheimer's Disease Amyloid-beta Oligomerization and Physiological Activity

Tissue & Cellular Engineering

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Abstract

Alzheimer's disease (AD) is the most common form of dementia, characterized by brains that have developed extracellular plaques composed of aggregated amyloid-beta protein. Mounting evidence suggests that the plaques themselves do not induce neurodegeneration; it is instead induced by amyloid-beta oligomers, which are formed in an intermediate step of plaque formation. Thus, one therapeutic goal is to prevent nascent amyloid-beta monomers from aggregating into toxic oligomers. Unfortunately, the current diagnostic criteria for AD do not present in early stages of the disease when intervention can be successful. As a result, naturally occurring compounds that can inhibiting Aβ oligomerization would be the ideal therapeutic as they can be safely incorporated into a population's diet in a strategy similar to iodized salt. By taking advantage of differences in diets between cultures, epidemiological studies have demonstrated a correlation between the increased intake of polyphenols and reduced incidence of AD. Many fruits, vegetables, and cereals contain flavonoids, the most common polyphenolic compounds found in the human diet. The most basic flavonoid is the three aromatic ring structure, flavone. While many flavone derivatives exist, this study focuses on the base structure and two derivatives with hydroxyl functionalized aromatic rings: 3',4'-dihydroxyflavone (DHF) and 5,7,3',4'5'-pentahydroxyflavone (PHF). We explored the therapeutic potential of these three compounds by examining their ability to inhibit amyloid-beta oligomerization and physiological activity. SDS-PAGE and Western blot analysis demonstrated that the presence of DHF and PHF reduces the maximum size of detectable amyloid-beta oligomers. In parallel experiments, these two polyphenols were found to have opposing effects on oligomer structure, evidenced as exposed hydrophobic domains using the hydrophobic dye ANS, with

PHF dramatically increasing oligomer hydrophobicity. Using the neuroblastoma cell line SH-SY5Y, oligomers formed in the presence of both DHF and PHF induced a less pronounced activation of NF κB, which is associated with neuronal dysfunction and toxicity. Together, these results demonstrate that polyphenols have the potential to modulate amyloid-beta oligomer formation and physiological activity, and that reducing oligomer size is more important than inducing changes in oligomer surface structure in the prevention of AD pathology.

IBE Abstract # 531

An Agent-based Model of Ductal Carcinoma in situ (DCIS) and its Validation in a Tissue-engineered Model of DCIS

Tissue & Cellular Engineering

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Abstract

Breast cancer often begins in the breast as ductal carcinoma in situ (DCIS). DCIS is the most common precursor to invasive ductal carcinoma (IDC) that is considered the second main cause of death in women in the States. The tumor and its microenvironments including biochemical and biomechanical factors interact with each other and these interactions play a primary role in tumor initiation, growth, and metastasis. These interactions consist of complex relations between tumor cells, soluble factors, and stromal components such as fibroblasts, and extracellular matrix (ECM) proteins. Understanding these relationships may lead to new therapeutic approaches to breast cancer. To improve the understanding of these relations and the progression to metastasis, we have developed a 2D agent based model of DCIS growth and progression to IDC, and compared this with tissue-engineered model of DCIS.

IBE Abstract # 464

Zein: New polymer for nonviral gene delivery

Tissue & Cellular Engineering

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Abstract

Many polymers have been used for nonviral gene delivery, but all fall short of successfully delivering DNA and subsequently expressing the protein encoded at an efficient amount for therapeutic use. Zein, a storage protein from corn, has unique biological and physical properties that make it a promising new polymer for gene delivery. Our objective was to investigate the novel use of zein and its interaction with DNA in two forms, nanospheres and films. We fabricated zein nanospheres encapsulating DNA through a simple coacervation technique and then characterized the spheres as a potential particulate delivery system for gene delivery. Zein/DNA nanospheres ranged from 100-400 nm in size and reached a maximum of 65% DNA encapsulation efficiency with a maximum loading of 6.1 mg DNA/g zein. The DNA within the zein spheres demonstrated resistance to degradation from DNase I and had a sustained release in PBS over 7 days. Cell studies showed robust biocompatibility and internalization of the particles, demonstrating the potential of these particles for DNA carriers, for applications including oral delivery. Zein was confirmed to be a new carrier and protector of DNA and a promising candidate for gene delivery. Zein was also fabricated as a film and investigated as a support for substrate-mediated gene delivery. Zein was dissolved in ethanol and films were formed by solvent casting. DNA complexes were absorbed to the surface of the zein film and cells were seeded. Zein films demonstrated transfection, and such films could be used for coatings on medical devices, to provide local delivery of therapeutic genes. Together, our nanoparticle and film studies reveal the versatility of zein to be used in gene delivery applications.

IBE Abstract # 481

Slanted Columnar Thin Film (SCTF) Substrates for Biomolecule Delivery and Cell Culture

Tissue & Cellular Engineering

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Abstract

Slanted columnar thin film (SCTF) substrates possess intricate sculptured features on the nanoscale with applications in photovoltaics, sensing (chemical, biological, optical, and pressure), micro- and nano-fluidics, biomaterials, and nanoelectronics, including field emitters, supercapacitors, and transistors. In this project, we have designed SCTF substrates with uniform slanting nanocolumns that are capable of loading biomolecules (proteins or DNA) within the intercolumnar void space of SCTFs. Using a novel combinatorial spectroscopic ellipsometry and quartz crystal microbalance with dissipation (SE/QCM-D) technique, we are able to demonstrate dynamic characteristics of biomolecule loading within SCTFs, including film thickness, adsorbed mass, and porosity with sub-angstrom resolution. Specifically, SE/QCM-D was used to analyze the adsorption of fibronectin (FN) and bovine serum albumin (BSA) within silicon and titanium SCTFs, which demonstrated that increased surface area of SCTFs significantly increases the loading capacity of proteins. Furthermore, both SE and QCM-D reported greater adsorbed mass and porosity for FN adsorption relative to BSA adsorption within SCTFs. We have also demonstrated the ability to functionalize individual silicon SCTF nanocolumns with stimuli-responsive PAA polymer brush coatings. SE data was collected and modeled before and after brush grafting. The ellipsometric model revealed polymer brush fractions ranging between 12-33% of the SCTF layer were present between the columns of the SCTFs. SEM was able to validate that polymer material penetrates within the intercolumnar void space of the SCTF film layer. Combinatorial SE/QCM-D measurements demonstrated the reversible, pH-dependent swelling characteristics of the polymer brushes, which could be used for biomolecule loading and release. Furthermore, we have been able to demonstrate

that SCTFs support robust cell adhesion and that DNA loaded within the SCTFs can be delivered to adhered cells. With such characteristics, SCTFs have the potential to impact the medical and biotechnological communities, including the fields of gene therapy, tissue engineering, biosensors, and diagnostics.

IBE Abstract # 482

Influence of alginate hydrogel biomechanical properties on the in vitro development of pre-implantation porcine embryos

Tissue & Cellular Engineering

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Abstract

In the pig, the pre-implantation period of pregnancy is highly influential on sow productivity and therefore the profitability of swine production. Between day 10 and 12 of gestation, the embryo undergoes a dramatic morphological change, known as elongation. During elongation the embryo produces and secretes estrogen, which serves as a key signal for maternal recognition of pregnancy. Deficiencies in elongation contribute to approximately 20% of embryonic loss, but exact mechanisms of elongation are poorly understood. In order to provide an in vitro tool for evaluating mechanisms of elongation, our objective was to utilize alginate hydrogels as three-dimensional scaffolds to encapsulate and support porcine embryo elongation in vitro. Specifically, we investigated biomechanical properties of alginate hydrogels of different concentrations and their effects on pre-implantation porcine embryo development. Embryos collected on day 9 of gestation were assigned to a 0.375%, 0.7%, or 1.5% alginate hydrogel system or to a control, non-encapsulated group and were cultured for five days. The Young's modulus of each alginate concentration was determined from compression tests in order to establish a direct link between matrix stiffness and embryo development. Varying the alginate matrix stiffness had a significant effect on embryo development throughout the culture period. These results demonstrate the role of the biomechanical environment on the in vitro development of pre-implantation porcine embryos and can be used to further optimize an in vitro culture system to facilitate and understand embryo elongation. Knowledge of the physiological mechanisms of elongation could lead to the identification of specific factors that could be targeted to improve pregnancy outcomes in the pig and other livestock species.

IBE Abstract # 504

Engineered B-Cell Biosensor for Specific, Sensitive and Rapid Detection of E. coli O157:H7

Tissue & Cellular Engineering

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Abstract

Foodborne pathogens are the major issue in food safety and rapid detection is needed to more effectively identify pathogens in food. The objective of this study was to develop an engineered B cell biosensor with better combined speed and sensitivity than current methods for rapid detection of E. coli O157:H7, one of the major foodborne pathogens. The biosensor was based on the genetically engineered B lymphocyte which was introduced with a pair of Fluorescence Proteins (FPs) and Calcium indicator. When E. coli O157:H7 attached to its specific receptors on the B cell surface, B cell receptors (BCR) induced Ca2+ signal pathway leading to Ca2+ flux. Then Ca2+ flux activated the FRET (fluorescence resonance energy transfer)-based Calcium indicator to report the fluorescence signal change and indicated the presence of E. coli O157:H7. A genetically encoded fluorescent reporter for Ca2+ (TN-XXL) was transfected into the B cell that consisted of fusions of cyan fluorescent protein (CFP), a troponin C-based Calcium indicator and yellow fluorescent protein (YFP). The proof of concept for the developed engineered B cell biosensor was conducted using E. coli O157:H7 at a concentration of 105 cfu mL-1. The fluorescence change of fluorescent proteins induced by FRET was observed.The ongoing research focuses on the optimization of biosensor parameters and then the evaluation of the developed biosensor for detection of E. coli O157:H7 in foods.

IBE Abstract # 526

Effect of Media Formulation on Human Mesenchymal Stem Cells (hMSCs) Maintenance In Vitro

Tissue & Cellular Engineering

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Abstract

Biomedical studies involving the use of bone marrow-derived human mesenchymal stem cells (hMSCs) is a rapidly-growing research area. hMSCs, which are harvested from bone marrow, must be expanded in vitro because of the small quantities of cells isolated. In both in vivo and in vitro environments, chemical cues affect stem cell proliferation and differentiation. Therefore, it is important to establish standard protocols for in vitro experiments, as chemical modifications to hMSC maintenance environments can alter long-term research results including differentiation studies. In this work, we investigate the effects of different media compositions on hMSCs throughout normal in vitro maintenance. hMSCs were cultured in mesenchymal stem cell growth media (MSCGM, Lonza), low glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) or DMEM supplemented with 10% FBS and 10-7 M dexamethasone (Dex). Cells were examined at passages 4, 5, 8 and 10. Changes in mRNA regulation, protein expression and proliferation were studied via quantitative real-time polymerase chain reaction (qRT-PCR), immunocytochemistry (ICC) and cell counts, respectively. Morphological differences were also observed over the course of the experiment. Results of ICC studies indicate changing levels of stem cell, osteogenic and myogenic protein expression throughout the passaging of hMSCs in all media treatments. Loss of STRO-1 was noted in all media treatments by passage 10. Osteonectin (ON) expression was detectable in all passages of cells treated with MSCGM and DMEM; decreased ON expression occurred in cells passaged in Dex media. Tropomyosin was observed in later passages with highest expression in DMEM-treated hMSCs. Regulation of mRNA expression varied widely as a function of media treatment and passage number. Cell proliferation changed in relation to passage number and media composition, including observable cell death in later

passages. Results of this study illustrate the dynamic response of hMSC maintenance to differences in growth medium and passage number. These experiments highlight the effect growth medium has on in vitro experiments and the need of consistent protocols in hMSC research.

IBE Abstract # 537

Human Mesenchymal Stem Cell Elastic Modulus directs Differentiation Capacity

Tissue & Cellular Engineering

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Abstract

Human mesenchymal stem cells (hMSCs) were passaged on tissue-culture polystyrene (TCPS) to investigate the *in vitro* aging and cell stiffening. TCPS, with an elastic modulus of approximately 3 GPa, is several orders of magnitude stiffer than physiological values, which are typically in the kPa range. It is known that hMSCs are subject to *in vitro* aging, which is hallmarked by telomere shortening, slowed proliferation, and decreased differentiation capacity. It has also been shown that hMSCs stiffen over many population doublings, most likely as a result of stiff culture substrates. It seems evident that cell culture conditions are contributing to the *in vitro* aging effects of cell stiffening and decreased differentiation capacity. To examine the effects of *in vitro* aging on differentiation capacity, osteogenic and myogenic differentiations were carried out at each passage. Local cell elastic modulus was measured at every passage using atomic force microscopy (AFM) indentation. Gene and protein expression was examined using quantitative real-time polymerase chain reaction (qRT-PCR) and immunofluorescent staining, respectively, for osteogenic (osteocalcin, osteonectin, osteopontin, alkaline phosphatase, Runx2) and myogenic (tropomyosin, sarcomeric actin, smooth muscle α actin, calponin 1, troponin T, desmin) markers. Cells passaged on TCPS were subject to cell stiffening. The elastic modulus of undifferentiated cells remained constant (approximately 8kPa) from P3 to P5. Significant increases ($P<0.05$) were observed from P6 (9.65±1.13 kPa) to P7 (12.97±1.26 kPa) and P8 (17.42±1.46 kPa). After P8, the average elastic modulus value was 19kPa. These increases in cell elastic modulus were accompanied by increases in actin stress fiber diameters. Elastic modulus values of hMSCs largely dictated the success of myogenic differentiation. Highest expression of myogenic markers were observed at P7, where E=12.97±1.26 kPa, which is comparable to previously reported values for muscle cells. Osteogenic

differentiations were more successful in earlier passages (P4 through P6), which may suggest that hMSCs more easily differentiate when in a softer state. This study gives insight into hMSC stiffening and corresponding differentiation capacity.

IBE Abstract # 538

Gold Nanoparticles Conjugated to Purified Collagen

Tissue & Cellular Engineering

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Abstract

Due to its many advantageous properties, collagen has been utilized as a scaffold for tissue engineering applications. However in its purified state, collagen is mechanically weak and will undergo rapid degradation. To mitigate these effects, collagen can be crosslinked. While enhanced mechanical properties and stability can be achieved by crosslinking, collagen can be rendered less biocompatible either due to changes in the overall microstructure or due to the cytotoxicity of the crosslinkers. We have investigated using gold nanoparticles (AuNPs) attached to collagen in order to enhance mechanical properties and resistance to degradation while also maintaining its natural microstructure and biocompatibility. Porcine collagen was crosslinked with AuNPs using a zero-length crosslinker, EDC. Several characterization studies were performed including electron microscopy, differential scanning calorimetry (DSC for thermal stability), collagenase assays, and biocompatibility assays. The results demonstrated that AuNP-collagen scaffolds had increased resistance to degradation as compared to non-AuNP-collagen while still maintaining an open microstructure. While the biocompatibility assays showed that the collagen and AuNP-collagen scaffolds are biocompatible, the AuNP-collagen demonstrated enhanced cellularity over the collagen scaffolds. Additionally, the DSC studies indicated the ability of the AuNP-collagen to enhance thermal stability. Overall, the AuNP-collagen scaffolds demonstrated enhanced biocompatibility and stability over non-AuNP scaffolds.

IBE Abstract # 544

Methods for in vitro scaffold free cartilage tissue engineering

Tissue & Cellular Engineering

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Abstract

Rationale for the research: Articular cartilage has an extremely low capacity for intrinsic repair, and cartilage injuries often progress to debilitating osteoarthritis. Cartilage tissue engineering generally involves the implantation of cells, scaffolds, or a combination of the two with the aim of alleviating pain by regenerating functional and durable tissue.

Objectives: The objective of the current research is to develop a reliable method for in vitro scaffold-free cartilage tissue engineering based on high-density culture of mesenchymal stem cells in transwell culture inserts.

Methods:We tested several variations of the same basic system: Monolayer-expanded, primary human bone marrow mesenchymal stem cells (hbMSCs) were seeded at high density into 6.5mm diameter transwells and cultured for up to 28 days in defined chondrogenic medium which contained 10 ng/ml transforming growth factor beta-3 (TGF²-3). Chondrogenesis was assessed from construct size and appearance, DNA and glycosaminoglycan (GAG) contents, toluidine blue-stained histological sections, and immunohistochemistry to detect the presence of collagen type II. Several variations of the basic system were evaluated. These included the brand of transwell culture insert, the rate of transition from expansion to chondrogenic medium, cell seeding density, addition of fibroblast growth factor (FGF) to the medium, and collagen coating of the transwell membranes.

Results and Conclusions: The basic system did show that hbMSCs could be transformed to cartilage when seeded into a transwell at high density. The use of different transwell culture inserts did not show any significant changes in development of the cartilage constructs. The cell seeding density experiment did show that there is an optimal density that allows for optimized growth over time. The GAG and DNA content was roughly proportional to seeding densities, but GAG normalized to DNA peaked around the seeding density of 8 million. The 8 million seeding density was the intermediate seeding density between

4 million and 12 million. The use of FGF did not show any effects on the growth of the constructs. The collagen coating did show effect on the growth of the constructs. Some constructs did suffer from a balling effect. This balling effect caused the constructs to contract into a small ball on the membrane surface of the transwell instead spreading out over the whole area of the transwell. Various techniques were used to mitigate this effect. The two factors that reduced the effect of balling the most were seeding at a density of around 8 million cells and coating the membrane of the transwell with collagen.

IBE Abstract # 565

Pinewood activated char for mitigation of p-cresol

General Poster Session & Student Poster Session and Competition

Lalitendu Das, North Carolina State University; Dr. Praveen Kolar, North Carolina State University; Dr.John. J. Classen, North Carolina State University; Dr. Jason A. Osborne, North Carolina State University

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Abstract

There is significant interest in the synthesis of value-added products from lignocellulosic and agricultural residues. In this study K2CO3 assisted low-temperature carbonization process was explored to synthesize a low-cost adsorbent from pinewood for mitigation of p-cresol from water. A central composite design was employed to study the effects of carbonization time, impregnation ratio (IR), and carbonization temperature on adsorbent yield and adsorption capacity. Optimum adsorbent yield of 63.22 % and adsorption capacity of 5.40 mg g-1was obtained at a carbonization temperature of 266 °C, IR of 2, and carbonization time of 2 h Theoretical adsorption capacity of 6.97 mg g-1 was observed at 25 °C, unadjusted pH and an adsorbent dose of 10 g L-1. In addition, kinetic studies indicated that chemisorption was the predominant mechanism. Further, adsorption of p-cresol decreased in presence of surface acidic oxygen groups, and with increase in temperature due its exothermic nature. Results are expected to decrease odor related problems, enhance the image of swine industry, and add value to pinewood.

IBE Abstract # 440

Investigation of Media Ingredients and Water Sources for Algae CO2 Capture at Different Scales to Demonstrate the Correlations Between Lab-scale and L

General Poster Session & Student Poster Session and Competition

Tabitha Graham, University of Kentucky Biosystems and Agricultural Engineering, Czarena Crofcheck,University of Kentucky Biosystems and Agricultural Engineering, Aubrey Shea,University of Kentucky Biosystems and Agricultural Engineering, Michael Montross,University of Kentucky Biosystems and Agricultural Engineering, Mark Crocker, University of Kentucky Biosystems and Agricultural Engineering

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Abstract

As energy use increases globally the environmental burdens increase alike. Many accusations have been made that carbon dioxide is a culprit of climate change. The University of Kentucky and Duke Energy Power have partnered to test carbon capture technology in a large scale project. The objective is to investigate potential water media sources and nutrient sources at different volume scales for algae cultivation to help create a more environmentally viable and economical feasibility. This work will analyze a life cycle assessment of water media sources and the effects of the inputs and outputs needed for each medium. The up-scaling objective of the research is to identify what parameters vary as a result to up-scaling and how to maintain a culture at the large scale that is standardized to the lab scale culture.

was dependent on the treatment time and concentration of the NPs. However, the mechanism of the inhibitory effect is not clear yet, and needs further research.

IBE Abstract # 551

Effect of Gold/Copper Sulfide Core/Shell Nanoparticles on Bacillus Anthracis Spores

General Poster Session & Student Poster Session and Competition

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Abstract

Bacillus anthracis is a gram positive, spore forming bacteria that is known to cause the anthrax disease. In 2001, the spores of B. anthracis were used in the bioterrorism attack in which five people died. B. anthracis spores are more resistant to antimicrobial agents than their vegetative cells. Metal nanoparticles (NPs), which have been previously shown to have antimicrobial effects against vegetative microbes, have great promise in having similar effect against the more resistant spores. In this work, we studied the effect of gold/copper sulfide (AuCuS) NPs on the germination and outgrowth of B. anthracis spores. When B. anthracis spores at concentration of ~5 x 108spores/ml was pre-treated with 0.0083, 0.083, 0.83, 1.66 and 4.15 µM of AuCuS NPs at room temperature for 30 min, a concentration dependent inhibition of B. anthracis growth by the AuCuS NPs was observed. The results showed that, treatment with AuCuS NP concentrations 0.83 µM, 1.66 µM and 4.15 µM completely inhibited spore growth whiles spores treated with 0.0083 µM and 0.083 µM NPs prolonged their lag phase to two and six hours, respectively. Without 30 min pre-treatment, immediate growth test after the addition of NPs to spores showed the same results, indicating that the continuous presence of NPs during incubation but not the 30 minutes treatment was responsible for inhibition of spore germination by 0.83 µM AuCuS NPs. To examine the effect of pre-treatment time, spores were treated with NPs for 0.5, 3, 6 12 and 24 hours, the plating results showed a time and concentration dependent viable spore number reduction. Pretreatment with 0.83 µM and 4.15 µM NPs for 12 hour reduced viable spore number by approximately 0.6 log and 1 log, respectively. We present here that, AuCuS NPs showed inhibitory effect on B. anthracis spores, and its inhibitory effect

IBE Abstract # 550

A Nanomaterial-Mediated Biosensor for Measuring Sarcosine

General Poster Session & Student Poster Session and Competition

Grace Justinvil, University of Florida; Stephanie L. Burrs, University of Florida; Diana Vanegas, University of Florida; Eric S. McLamore, University of Florida

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Abstract

Prostate cancer is the second most common cause of death in men in the United States. Prostate cancer is difficult to detect due to low-grade prostate cancer cells. Sarcosine, a non-essential amino acid found in human urine and blood serum, has been identified by researchers as a potential biomarker for prostate cancer; sarcosine concentrations in human tissue samples have been found to increase during prostate cancer progression. The use of sarcosine as a biomarker has potential to improve detection and to complement the standard Prostate Specific Antigen (PSA) test. Sarcosine biosensors enhanced with electrocatalytic nanomaterials potentially offer accurate, rapid, and inexpensive detection in human urine samples. In this research, sarcosine biosensors were fabricated using platinum nanoparticles (platinum black), single-layered graphene oxide, and sarcosine oxidase. Sarcosine oxidase catalyzes the oxidation of sarcosine to a formaldehyde and hydrogen peroxide. The peroxide decomposes to hydrogen, water, and two electrons; this current is measured at the surface of the electrode via amperometry. Biosensors were analyzed using DC potential amperometry (DCPA) and cyclic voltammetry (CV) tests. CV tests were used to determine electroactive surface area of the nanomaterial-mediated biosensor via the Randles-Sevcik equation. DCPA was used to quantify sensor sensitivity, selectivity, response time, hysteresis, and operating range. The sensitivity was 5.8 ± 3.2 nA μM^{-1}. The lower limit of detection was 0.54 ± 0.093 µM. Ongoing work is being conducted to quantify the other performance characteristics.

IBE Abstract # 546

Agent-Based Models for Synthetic Biology

General Poster Session & Student Poster Session and Competition

Laurie J. Heyer, Davidson College; Andrew Lantz, Davidson College; Tucker Whitesides, Davidson College; Jonah Galeota-Sprung, Davidson College; Todd T. Eckdahl, Missouri Western State University; A. Malcolm Campbell, Davidson College; Jeffrey L. Poet, Missouri Western State University

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Abstract

Synthetic biologists often need to model an interacting network of molecules to guide the design of an engineered biological system. Classical models use systems of differential equations to represent transcription, translation, and other biological processes. However, these models assume large quantities of each molecular species, uniformly mixed in solution, and do not accurately represent small numbers of molecules or the random variation that may be key to system function. Even if the modeler is willing to accept these limitations, large systems of equations are cumbersome to build and analyze, particularly when many parameter values are unknown. Although stochastic event simulations (e.g., the Gillespie algorithm) are more accurate for small numbers of molecules, this approach still assumes well-mixed reactants. A better approach for many synthetic biology scenarios is a simulation that tracks the location and state of individual molecules, or groups of molecules. Because of their emphasis on emergent properties arising from simple interactions, agent-based models (ABMs) are well suited to the modeling of complex biological systems such as gene networks. We demonstrate how to model synthetic gene circuits using the ABM paradigm, in which each molecule (or group of molecules) moves and interacts with others molecules according to a simple set of rules. In particular, we have implemented well-known systems, such as the Repressilator and Biopixel oscillator, using the freely available software NetLogo. NetLogo is open source, runs on all platforms, and resulting models can be easily integrated into web pages.

IBE Abstract # 542

Sustainable Green Roof Irrigation using Wastewater

General Poster Session & Student Poster Session and Competition

Samuel Frey, Environmental Engineering Department, University of Connecticut; J. Suen, Agricultural and Biological Engineering Department, University of Florida; R. Munoz-Carpena, Agricultural and Biological Engineering Department, University of Florida; E.S. McLamore, Agricultural and Biological Engineering Department, University of Florida

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Abstract

Water and energy are critical resources. Technologies such as green roofs reduce stress on infrastructure and create building energy savings by insulating heating/cooling systems. Onsite (i.e., decentralized) reuse of wastewater for irrigation of green roofs is a sustainable option that would reduce water demand and provide additional economic incentive. Inclusion of sustainable materials such as biochar improves retention of metals and ensures that green roofs are not point sources of pollution. A controlled study was conducted to examine the possibility of irrigating extensive (shallow) green roofs with air conditioner condensate (ACC), and the effect(s) of including biochar in the soil media on runoff water quality. Twelve plant beds (18□ X 25.5□ X 5) were built with well-drained, lightweight material with adequate water holding capacity; 45% perlite, 25% play sand, 15% vermiculite, 10% peat moss, and 5% compost. Six of the beds were seeded with biochar produced from pyrolysis of bamboo. One set of beds (three with biochar and three without biochar) containted Coreopsis, Gaillardia Pulchella, and Frostweed. The other six beds were planted with a common grass. ACC production was measured daily and a basic psychometric model was constructed to estimate ACC production rate based on local weather data and building dimensions. Irrigation and runoff water quality, plant growth, and soil properties were measured to determine the effects of wastewater irrigation on plant health and also to assess potential risks to public health. The psychrometric model was capable of predicting ACC production rate; there was no significant difference between measured and predicted ACC production rates ($p=0.957$, $\pm=0.05$). The sodium absorption ration (SAR), electrical conductivity (EC), pH, total chemical oxygen demand (COD), and ion concentrations of the runoff water did not show any significant difference between different types of irrigation water. The pH of the ACC runoff water increased across the plant beds.

H2 where as the main treatment of interest for further study, the peach/soybean meal treatment, produced an average of 26.6mmol/L of H2. Acetate is another product of the T. neapolitana fermentation. It is an important as it is a relatively high-valued product that would be useful in industrial scale fermentations. It was found that on average the three peach treatments produced more (0.69g/L) acetate than the four glucose treatments (0.62g/L). The third major product is carbon dioxide. The peach treatments were found to produce on average, slightly more carbon dioxide than the glucose treatments. Overall, the treatments were found to produce the 1:2:1 ratio of acetate, hydrogen, carbon dioxide, respectively, per mol of glucose that is expected from the stoichiometry. It can be concluded that using peaches along with soybean or canola meal is a feasible replacement for the glucose and yeast media for T. neapolitana. It has also been concluded that the peach/soybean meal treatment is the preferred media to use in further studies.

IBE Abstract # 540

The Effect of Agricultural-Based Nitrogen Sources on Production of Biohydrogen by Thermotoga Neapolitana

General Poster Session & Student Poster Session and Competition

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Abstract

Alternative fuel sources are needed to provide cleaner energy for our world. Many alternatives exist, with one of those being hydrogen. Hydrogen gas can be produced by some micro-organisms that use special metabolic pathways. Thermotoga neapolitana is a fermentative hyperthermophile that was found in the Bay of Naples over 25 years ago. T. neapolitana has been found to produce hydrogen as a product of its anaerobic fermentation of glucose. Using a prescribed method for making the media Thermotoga neapolitana was grown using agricultural-based carbon and nitrogen sources. The standard media for T. neapolitana requires glucose as the carbon source and yeast extract and trypticase as the nitrogen sources. Various agricultural feedstocks have also been investigated to determine their viability as carbon and nitrogen sources for these fermentations. Each year in South Carolina alone, 20 million pounds of peaches are discarded because of imperfections. These peaches contain glucose, fructose and glucose which makes them an option for a carbon source. Soybean meal and canola meal are local, waste feedstocks that have potential as nitrogen sources. The experiment conducted was a comparison of hydrogen production by T. neapolitana in batch fermentation for seven different carbon/nitrogen source treatments: the standard media mention above, glucose/yeast, glucose/canola meal, glucose/soybean meal, peaches/yeast, peaches/canola meal, peaches/soybean meal. These bottles were sparged with nitrogen, inoculated and were placed in a fermentor/shaker bed for 40 hours at 77°C. The pressure in the bottles was measured followed by analysis on a gas chromatograph to determine the percentage of each gas in the headspace of the bottles. Next, liquid chromatography analysis was done on pre and post fermentation media to determine sugar and acetate concentrations. After all analysis was complete it was determined that the hydrogen production (in mmol/L) from the seven treatments is not significantly different despite the standard media having higher hydrogen yields. The standard media produced an average of 26mmol/L of

Given an asRNA sequence, the binding percentage is calculated by NUPACK software suite. This correlation serves as a predictive model that enables designing asRNA sequences as adjustable switches to control the expression of target genes inside a cell. One notable long-term industrial application of this research would be targeted product optimization in response to specific bioreactor conditions by controlling the metabolic flow through certain pathways.

IBE Abstract # 533

Fine-tuning Bacterial Gene Expression using Antisense RNA

General Poster Session & Student Poster Session and Competition

Hadi Nazem-Bokaee, Virginia Polytechnic Institute and State University; Ryan S. Senger, Virginia Polytechnic Institute and State University

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Abstract

Regulation of gene expression is one of the highly complex mechanisms by which cells can adapt to new food sources, respond to environmental perturbations, and control their gene functions. Although there is an ongoing research on this area in biology, the complete understanding of details of gene expression regulation mechanisms in different biological systems has not yet been achieved. Antisense technology has been developed over the past three decades as one of the most powerful research tools for the analysis of gene function. However, the majority of focus has been on studying the gene function in eukaryotic cells. Therefore, more investigations on gene regulation and function of bacterial systems using antisense technology will extend our knowledge on the physiology of bacterial cells and further expand their commercial applications. In this work, we applied the Antisense RNA (asRNA) technique to a bacterial system by which controlled gene knock-downs were enabled. One of the advantages of asRNA technique among other antisense techniques is the possibility of in vivo expression of the target asRNA through delivery of expression vectors to bacterial hosts. Antisense RNA (asRNA) molecule is a single-stranded RNA molecule that binds to complementary mRNA and blocks the translation by the ribosome. To study the gene down-regulation caused by asRNA, we constructed several reporter gene systems. These reporter systems are essentially vectors containing different fluorescent protein genes (excitation from 450 to 650 nm) and are replicated in E. coli cells. Using fluorescent proteins allows both qualitative and quantitative analysis of the gene function in vivo. asRNA fragments were designed complementary to the sequence of different regions of these fluorescent protein genes and were cloned into the reporter systems under the control of an inducible promoter. A BioTek synergy H4 Hybrid Multi-Mode Microplate Reader was used for real-time monitoring the growth of E. coli cells expressing the fluorescent proteins to quantitatively measure the expression level (fluorescent intensity). The collected data on the expression levels of different fluorescent protein genes are correlated to the percentage of asRNA binding mRNA.

IBE Abstract # 532

Phycocyanin Production by Cyanobacterial Biofilms Cultured in Oilfield Wastewater (Produced Water)

General Poster Session & Student Poster Session and Competition

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Abstract

Phycocyanin is a high value pigment that may be used for a variety of applications. Potential uses include immunoassay markers, dyes, and as feedstock for pharmaceutical and nutraceutical preparations such as mesobiliverdin, a cytoprotective anti-inflammatory agent that is a close homolog in structure to biliverdin. The objective of this research was to produce phycocyanin using cyanobacterial biofilms cultured on oilfield wastewater (produced water) to bioremediate the wastewater and decrease disposal costs while producing the high-value pigment. Cyanobacterial biofilms were cultured utilizing Rotating Algal Biofilm Reactors (RABRs) in full strength produced water and harvestable biomass and extracted phycocyanin yields were determined. RABRs operating in Produced Water Medium (PWM) yielded 9.43g AFDW cyanobacterial biomass per square meter and 11.2mg phycocyanin per gram AFDW biomass. It is concluded that produced water is a suitable medium for cyanobacterial growth and phycocyanin production utilizing the RABR platform.

that the use microbes for dispersant-removal (a natural, non-toxic removal method) would enhance Gulf remediation efforts.

IBE Abstract # 528

A Nano-Zeolite Sensor to Detect Surfactants, a Contribution to Microbial Remediation Feasibility Studies

General Poster Session & Student Poster Session and Competition

Katelyn S. Ward, University of Florida; Dr. Eric S. McLamore, University of Florida; Prachee Chaturvedi, University of Florida; Stephanie Burrs, University of Florida; Shige Taguchi, University of Florida; Diana Vanegas, University of Florida

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Abstract

After the 2010 Deepwater Horizon oil spill, the need for innovative restoration processes has become urgent in order to avoid ecological and economic distress. One of the most well-known techniques involves the distribution of dispersants that break down oil into water-soluble micelles, but may also be harmful to the Gulf ecosystem.While microbes such as Pseudomonas aeruginosa can effectively metabolize hydrocarbons that have negatively affected Gulf wildlife, the environmental threats associated with dispersants are less readily apparent than those of the oil. Experiments have shown that dispersants leave dangerous levels of hydrocarbons in fish (often killing the fish eggs) and can cause reproductive anomalies in clams. One technology that is currently under development for improved cleanup of oil spills involves nutrient-infused aerogels seeded with enriched oil-degrading microbes. The use of these natural microbes is an innovative approach to reducing dispersant levels without adding an additional inorganic ingredient to the cocktail of chemicals poured into the Gulf to counter oil spills. While evidence shows that oil uptake through oleophilic nano-channels and subsequent degradation is a promising technology for rapid spill mitigation, the effects of dispersants on this readily deployable solution is unclear. The overall objective of this research is to build and test a sensor which will detect characteristic molecules in dispersants used during the cleanup of oil spills. This sensor will be combined with existing physiological sensors to measure Oxygen and Iron levels, in addition to H+, and Ca2+ flux in lab studies designed to determine the feasibility of using aerogel-infused microbial remediation for hydrocarbon degradation. The significance of this sensor is two-fold: it creates concrete justification for bioremediation techniques that may be costly or time-consuming, and specifically supports the premise

and also their hydrophobicity differences, molecular sieve or zeolite-based membrane systems were tested.

IBE Abstract # 524

Solvent Selection and Recovery for Liquid-Liquid Extraction of Acetic Acid and Water

General Poster Session & Student Poster Session and Competition

Mahdieh Aghazadeh, Purdue University; Abigail Engelberth, Purdue University

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Abstract

The removal of acetic acid from biomass hydrolysate is crucial to increase the yield of ethanol during fermentation. Acetic acid is a well-known inhibitor and is present in all biomass in its native form as an acetyl group. The acetyl groups are released from the biomass during hydrolysis as acetic acid. Hydrolysis is a processing step in which the long-chain sugars in the biomass are broken into smaller chains that can be consumed by the yeast to form ethanol. The acetic acid released during hydrolysis works to slow down the fermentation and significantly decrease the yield of ethanol. The overall goal of this project is to explore the removal of acetic acid from the hydrolysate using liquid-liquid extraction (LLE). The use of liquid-liquid extraction to remove acetic acid from water is traditionally performed with ethyl acetate. This step is then coupled to a distillation tower to purify the acetic acid from the solvent stream. Distillation also allows the ethyl acetate to be reused in the LLE column. The drawback to this method is the operational expense of the distillation column. The use of a membrane for separation is more cost effective and possibly even more efficient. The work performed for this study was to use Aspen Plus to determine a reasonable solvent to recover Acetic Acid from water. A list of solvents was identified based on the tertiary-phase diagram with acetic acid and water. The phase diagrams demonstrate how a mixture of three solvents forms either a single or biphasic system. Next, the solvents that were available in the Aspen Plus data bank were simulated to test how well they remove acetic acid from water. The aim was to identify a solvent that has great affinity to acetic acid and low solubility in water (i.e. a large partition coefficient), and also required the least extreme operating conditions as far as temperature and pH were concerned. Once the solvent was identified, the next step was to determine the best type of membrane. Based on the molecular size of the solvent and acetic acid

IBE Abstract # 514

Isolation and characterization of anaerobic microorganisms from the Logan City Wastewater Lagoon System for the production of high value bioproducts

General Poster Session & Student Poster Session and Competition

Joshua Ellis, Neal Hengge, Ronald C. Sims, and Charles D. Miller, Utah State University

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Abstract

The ability to engineer novel systems for the production of high value products such as biosolvents, bioacids, and biogas from cheese whey, a waste feedstock rich in lactose and protein, has been demonstrated. Several clostridia species were isolated from the anaerobic sediments of the Logan City Wastewater Lagoon System. Isolates were obtained by counter-selecting against non-spore forming bacteria, diluted to extinction, and streaked for isolation several times to ensure purity. Isolation was confirmed by amplifying the 16S rRNA subunit and comparing the sequences to the NCBI database. Phylogenetic analysis of these isolates indicates that they are closely related taxonomically to Clostridium butyricum, Clostridium metallolevans, and Clostridium bifermentans, while numerous others were not phylogenetically similar to characterized organisms within the NCBI database indicating that these isolates may indeed be novel. The production of high value bioproducts using cheese whey has been quantified using GC and HPLC. Initial studies have shown hydrogen production yields to be 1.2 mol-H2/mol-lactose, while ethanol production was 2.1g/L, with a volumetric ethanol productivity of 0.058 g/L, as well as the production of lactate, butyrate, and acetate from certain isolates. Our goal is to demonstrate the feasibility of understanding relationships within a complex anaerobic community to aid in isolating microbes with physiologies of interest to engineer novel strategies for the production of high value and renewable bioproducts.

IBE Abstract # 511

Characterization of the Pradimicin A Biosynthetic Pathway

General Poster Session & Student Poster Session and Competition

Kandy Napan, Department of Biological Engineering, Utah State University; Whitney Morgan, Department of Biological Engineering, Utah State University; Thomas Anderson, Department of Biology, Utah State University; Jon Takemoto, Department of Biology, Utah State University

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Abstract

Pradimicin A is a potent antifungal and antiviral natural product. The late biosynthetic steps in pradimicin biosynthesis are not well understood. In this study, we investigated the pradimicin biosynthetic pathway through combinatorial biosynthesis and gene disruption approaches. We have obtained four new pradimicin biosynthetic intermediates, pKN92, KN90, KN87, and KN82, which for the first time confirmed the functions of pdmW and pdmS and revealed the order of four important tailoring reactions in the pradimicin biosynthetic pathway. This work allows us to understand pradimicin biosynthesis and further engineer the biosynthesis of new pradimicin analogs with improved therapeutic characteristics.

IBE Abstract # 510

Characterization of the herboxidiene biosynthetic gene cluster in Streptomyces chromofuscus ATCC 49982

General Poster Session & Student Poster Session and Competition

Jia Zeng, Department of Biological Engineering, Utah State University; Lei Shao, Department of Biological Engineering, Utah State University; Jiachen Zi, Department of Biological Engineering, Utah State University; Jixun Zhan, Department of Biological Engineering, Utah State University

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Abstract

We have recently sequenced the genome of Streptomyces chromofuscus ATCC 49982, the producer of the anti-cholesterol natural product herboxidiene. A 53-kb biosynthetic gene cluster was discovered and confirmed to be responsible for the biosynthesis of herboxidiene through gene inactivation. In addition to herboxidiene, a biosynthetic intermediate, 18-deoxy-herboxidiene, was also isolated from the fermentation broth of S. chromofuscus ATCC 49982 as a minor metabolite.

IBE Abstract # 501

Antisense RNA: A Metabolic Switch for Controlling the Gene Expression

General Poster Session & Student Poster Session and Competition

Hadi Nazem-Bokaee, Virginia Tech; Ryan S. Senger, Virginia Tech

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Abstract

Cell machinery in bacteria employs a complex series of processes to ensure the survival of the cell in response to internal and external perturbations. Regulation at the translational level is one of the vital yet less-understood mechanisms inside bacterial cells. Antisense RNA (asRNA) molecules are single-stranded RNA molecules that bind to complementary mRNA molecules and block the translation of mRNAs. Using asRNA has attracted more attentions among scientists in recent years as an effective method for controlling microbial gene expression at the translational level. However, in most of the cases, the use of asRNA has been resulted in a complete knock-out. In this work we are interested in developing an asRNA system which allows controlled gene knock-downs. To monitor the reduction in expression levels, different fluorescent protein genes with excitation wavelength numbers spanning from 450 to 650 nm were used as reporter gene systems. asRNA fragments were designed complementary to the sequence of different regions of the fluorescent protein genes and were cloned into them under the control of lacZ promoter and terminator. A hybrid plate reader was used for real-time monitoring the growth of E. coli cells expressing the fluorescent proteins to quantitatively measure the expression level (fluorescent intensity). The ultimate goal is to correlate the protein expression level with the percentage of asRNA binding mRNA given an asRNA sequence. This method enables designing asRNA sequences as fine-tuning switches to control the expression of proteins inside a cell. One notable industrial application of this research would be in adjusting the metabolic flow through certain pathways in response to specific bioreactor conditions.

as a detector for chemicals, such as emerging contaminants, that may not have had their water quality criteria defined yet.

IBE Abstract # 497

Oxygen consumption as a rapid bioindicator of changes in water quality using Daphnia magna embryos

General Poster Session & Student Poster Session and Competition

Matthew Stensberg (Presenting), Purdue University; Michael Zeitchek, Purdue University; Kul Inn, Purdue University; Eric McLamore, University of Florida at Gainesville; Maria Sepulveda, Purdue University; D. Marshall Porterfield (Corresponding), Purdue University

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Abstract

Currently there are a variety of analytical chemistry based water quality monitoring systems which are capable of measuring low level pollutants. However, most are not readily field deployable and in those that are, their sample collection strategies are not optimized. We have developed a biological based detector of changes in water quality that could be used solely as a detector or used as a trigger system to be coupled with traditional field water samplers. The purpose of our research is to determine whether oxygen consumption in Daphnia magna embryos can serve as a suitable indicator of changes in water quality. For this experiment we examined an array of chemicals which have differing mechanism of toxicity: potassium cyanide, cadmium chloride, atrazine, malathion, and pentachlorophenol. Concentrations used were based on the EPA acute and chronic ambient water quality guidelines for the protection of aquatic life. Effects were first monitored by measuring oxygen flux at the surface of the embryos during exposure, then the experiment was scaled up to optically measuring oxygen concentration change in the microenvironment (modified microtiter plate) around the embryo to determine if the technique could be used in a high throughput system. All chemicals elicited a significant change in oxygen consumption in less than 2 hr during flux experiments, an improvement over 6-48 hr response times observed in other biological based monitoring systems. On the platform system, we demonstrated the ability to detect both an increase and decrease in oxygen consumption in embryos exposed to differing levels of potassium cyanide, again in less than 2 hr. These results indicate that D. magna embryos serve as a suitable bioindicator species for rapid detection of low-levels of contaminants in water. Because of this response to this broad array of chemicals, our results imply that oxygen consumption may also serve

in FbFPs through model-guided protein engineering. In this way, we adopt a comprehensive approach to understand and improve FbFP-based imaging technology. We anticipate that our work will enable the broad application of FbFPs and their bright mutants as reporters of gene expression, protein localization, and whole cell microbial sensors in conditions where GFP-based reporters fail to perform optimally (e.g., fast time-scale biological processes, extremophilic microbes, anaerobiosis).

IBE Abstract # 484

Development of Flavin-based Fluorescent Proteins for Biological Imaging

General Poster Session & Student Poster Session and Competition

Arnab Mukherjee(presenting), Department of Chemical and Biomolecular Engineering; Kevin B. Weyant,Department of Chemical and Biomolecular Engineering; Joshua Walker,Department of Chemical and Biomolecular Engineering; John Ossyra, Department of Agriculture, Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign

Corresponding Author: Charles Schroeder, Assistant Professor, Department of Chemical and Biomolecular Engineering, 107 Roger Adams Laboratory, 600 S Mathews Ave, Urbana, Illinois 61801

Abstract

In this work, we report on the development of flavin-binding photosensory proteins as new fluorescent reporters with potential applications in synthetic biology and biological engineering. Flavin-based fluorescent proteins (FbFPs) were recently reported as novel reporters that are characterized by small size and oxygen-independent maturation of fluorescence, which are key advantages over the widely used green fluorescent protein (GFP). However, FbFPs are at a nascent stage of development and a full understanding of the performance and properties of FbFPs as a practical set of biological probes is lacking. Therefore, we extensively characterized the existing set of FbFPs in vitro in terms of brightness, oligomeric state, maturation time, fraction of (fluorescent) holoprotein, pH tolerance, redox sensitivity, and thermal stability. Overall, FbFPs show important advantages as broad-spectrum biological reporters, including robust fluorescence over a broad pH and temperature range (pH 4-11, up to 60C) and rapid maturation of fluorescence (< 3 min. vs. 30 min. in GFP). In addition, we validated FbFPs as stable fluorescent reporters in vivo by constructing a series of FbFP-based transcriptional constructs to probe promoter dynamics in Escherichia coli (see attached figure 1). Furthermore, we applied directed evolution and isolated two FbFP mutants that show a 2-fold enhancement in fluorescence emission over the parent protein (see attached figure 2). Finally, we are developing a molecular framework to computationally focus identification and engineering of FbFPs with novel properties. In particular, we are developing a 'molecular fingerprint' for FbFPs by integrating structural, thermodynamic, and evolutionary data on amino acids comprising the flavin-binding pocket in homologous proteins (see attached figure 3). Based on the fingerprint, we predict a mutational phase space for improving fluorescence and thermal stability

IBE Abstract # 477

Nature-inspired porous silica biomaterials for precision size exclusion at the mammalian cell surface

General Poster Session & Student Poster Session and Competition

Jennifer L. Kahn, Purdue University; Jenna L. Rickus, Purdue University

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Abstract

The ability to design molecular weight cutoff levels for a precision size exclusion material at the cell surface would enable researchers to accurately control mass transfer across the cell membrane as well as enhance the viability of cell transplant therapies through protection from immune response. Materials for precision size exclusion at the nanometer to micrometer scale require a uniform pore size distribution across the entire surface. Size exclusion chromatography is a well-defined analytical technique and achieves desired precision with columns packed with porous silica. Due to its availability and biocompatibility, silica is a natural choice for size exclusion at the cell surface, but the methods used to create SEC materials are too harsh to be appropriate for living systems. Diatoms and sponges, however, create intricate patterns of porous biosilica with uniform pore distributions at the surface of their cells using silica that is present in their seawater environments. These biosilica materials provide structure as well as mechanical protection for the cells encased within, while allowing necessary flow of nutrients and wastes in and out of the cell. Using biological elements of the systems employed by both diatoms and sponges, we aim to develop biocompatible porous silica materials for precision size exclusion at the cell surface. Our investigation is focused on diatom silaffin proteins, sponge silicatein proteins, long-chain polyamines (LCPAs), and phospholipids. Characterization of surface properties, including microstructure morphology and pore size distribution, is determined by scanning electron microscopy (SEM) imaging. Collagen layers are exposed to purified diatom and sponge molecules involved in silica morphogenesis and imaged. Silica-depositing biomolecule combinations that have produced promising surfaces on collagen will be further explored using MIN6 pancreatic beta cells as an in vitro cell model. In addition to SEM imaging, physiological flux measurements will be used to evaluate mass transfer across the coated cell membrane.

into fibers. From the constructed parts, 16 highly characterized genetic components were sent to the Registry of Standard Biological Parts. Over 64 BioBrick parts were assembled and all of these are available upon request.

IBE Abstract # 475

Arachnicoli: Production and Purification of Spider Silk Proteins in Escherichia coli

General Poster Session & Student Poster Session and Competition

Ryan Putman, Utah State University; Asif Rahman, Utah State University; Charles Barentine, Utah State University; Andrea Halling, Utah State University; Brian Smith, Utah State University; Federico Rodriguez, Utah State University; Kathleen Miller, Logan, Utah State University

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Abstract

Spider silk is a biomaterial with extraordinary physical properties. The combination of tensile strength, elasticity, and even biocompatibility has opened eyes to a wide range of potential applications. A few implementations of spider silk may one day include: medical advances (skin grafts, biomedical sutures, and artificial tendons/ligaments), automotive safety (seat belts and airbags), and military applications (parachutes and body armor). However, the production of spider silk is not as simple as merely farming spiders and collecting the silk. Spiders are very territorial and cannibalistic, thus alternative means of production are necessary to generate enough spider silk for realistic use. The bacterium Escherichia Coli has been proven effective at producing this valuable product because of its manipulability and our intimate knowledge of the organism's genome and functions. Through the use of synthetic biology and molecular cloning techniques, recombinant DNA has been constructed and transformed into E. coli for production of synthetic spider silk. The goal is to take advantage of E. coli's ability to be used as a 'factory' for creating silk in a controllable and cost efficient system. Supplementation with additional tRNAs will be employed as a strategy to boost overall silk yield and extend cell viability. The creation of longer, repetitive spider silk genes will allow for a broad range of 'designer' silk to be synthesized and subsequently tested for increased mechanical properties. The spider silk gene sequence of the Argiope aurantia has been chosen because of the physical properties it displays as well as the relative lack of extensive studies.This year, the Utah State iGEM team has constructed the first spider silk BioBrick parts. A composite system was created from these parts, which allowed for the production of the spider silk protein in E. coli. After extracting and purifying this protein, synthetic spider silk was artificially spun

IBE Abstract # 473

Electroactive Polymer-based Nanocomposites For Multi-analyte Amperometric Biosensors

General Poster Session & Student Poster Session and Competition

Rajtarun Madangopal, Purdue University; Matthew C. Stensberg, Purdue University; Nicholas Pulliam, Purdue University; D. Marshall Porterfield, Purdue University; Jenna L. Rickus, Purdue University

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Abstract

Biosensing for physiology often requires simultaneous real-time measurement of multiple analytes within small spatial dimensions. Recent advances in nanomaterial synthesis and processing have led to a new generation of microelectrode-based sensors with high sensitivity and selectivity. However, traditional dip-coating and drop-coating methods cannot achieve site-directed transducer immobilization and currently limit the maximum attainable spatial resolution in multi-analyte sensing schemes. We have developed a novel three-step process for spatially controlled layer-by-layer assembly of enzyme-based amperometric biosensors using electrodeposited polymers and nanomaterials. We first establish the feasibility of this design by developing individual amperometric microelectrode sensors for glucose and lactate and then demonstrate the multi-analyte capabilities of such a scheme by fabricating a dual-microelectrode biosensor for simultaneous glucose and lactate sensing. Layers of nanostructured platinum, enzyme-doped conductive polymer poly(3,4-ethylenedioxythiophene) [PEDOT] and non-conductive polymer poly(o-aminophenol) [PoAP] were electrodeposited to achieve increased electroactive surface area, fine control over site and quantity of enzyme, and reduced interference. The layering and precise doping of these polymers allowed fine control of location and quantity of enzyme loading while simultaneously enhancing selectivity against known interferents. Deposition parameters for each layer were optimized to tune both the properties of the sensing surface and the interface to the transducer. The resulting sensors showed good linear range, fast response times, and negligible crosstalk when operated simultaneously in dual-electrode mode. These results validate the proposed multi-analyte sensor design and pave the way for simultaneous real-time monitoring of multiple analytes near cells and cell clusters, in both wire-based multi-electrode and lab-on-chip formats.

IBE Abstract # 468

Optrode biosensors for in vivo sucrose monitoring in plants

General Poster Session & Student Poster Session and Competition

Leyla Nesrin Kahyaoglu, Purdue University; Rajtarun Madangopal, Purdue University; Cliff Weil, Purdue University; Jenna L. Rickus, Purdue University

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Abstract

In higher plants, sucrose is the major type of carbohydrate produced by photosynthesis in leaves and is partitioned between the various sinks after its synthesis; however, what partitions the carbon to these various sinks remains unclear, particularly the essential first steps in sink establishment. Successful application of optical biosensors for the in vivo monitoring of sucrose in plants can facilitate our understanding of sucrose metabolism as well as improve breeding efforts. Optical fibers are excellent platforms for physiological applications, particularly for in vivo monitoring since fibers are non-electrical, thereby offering less invasive sensing. The objective of this work is to design and develop optrode-based sensors (fiber optic microprobes) through sol-gel and photopolymerization techniques to obtain in planta sucrose measurements over time. Optrode biosensors are constructed by pulling an optical fiber to micron-size diameter at the tip, and the tip is functionalized by adding a fluorescent sucrose indicator protein. Sol-gel and photo-initiated polymerization techniques are used and optimized to incorporate FRET-based sucrose sensors into a gel and polymer matrix. Photo-initiated sensor design based on several polymerization parameters including the monomer and photoinitiator concentration is optimized to ensure proper tip growth, improve doping efficiency, and maximize protein function. The sensors are characterized by using ratiometric binding isotherms at varying sucrose concentrations.

IBE Abstract # 465

Mesophilic Anaerobic Co-Digestion of Swine Manure with Swithgrass and Wheat Straw for Methane Production

General Poster Session & Student Poster Session and Competition

Zhimin Liu, North Carolina State University; Jorge Gontupil, North Carolina State University; Mr. Darwin, North Carolina State University; Angelica Pura, North Carolina State University; Jay J.Cheng, North Carolina State University

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Abstract

Anaerobic co-digestion of swine manure with switchgrass and wheat straw was studied for biogas production. The objective of the study was to investigate the effect of the addition of different percentages (2%, 4% and 6% % total solids (TS) based on agricultural residues (AR) on the yield of methane production. The inoculum (control) was cultivated in a completely-mixed and semi-continuously fed reactor. The reactor had a working volume of 14 liters and was operated at 35°C with a hydraulic retention time of 25 days and an agitation speed of 120 rpm. The reactor was fed with 560 ml swine manure and 14 g corn stover every day. Batch reactors were operated in triplicates, each with a working volume of 500 ml. Reactors were kept in respirometers under mesophilic conditions (35°C) with an agitation speed of 270 rpm. The volume of methane produced in experiment was measured by gas meters. COD, pH, TKN, %TS, %VS analysis were performed at the beginning and end of the experiment. The results indicate that with the addition of AR, the methane production was substantially enhanced.

IBE Abstract # 459

Economic production of Polyhydroxyalkanoates in Escherichia coli

General Poster Session & Student Poster Session and Competition

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Abstract

We have recently sequenced the genome of Streptomyces chromofuscus ATCC 49982, the producer of the anti-cholesterol natural product herboxidiene. A 53-kb biosynthetic gene cluster was discovered and confirmed to be responsible for the biosynthesis of herboxidiene through gene inactivation. In addition to herboxidiene, a biosynthetic intermediate, 18-deoxy-herboxidiene, was also isolated from the fermentation broth of S. chromofuscus ATCC 49982 as a minor metabolite.

IBE Abstract # 452

Evaluation of the Antimicrobial Properties and Biocompatibility of Polypropylene Mesh Conjugated with Gold Nanoparticles

General Poster Session & Student Poster Session and Competition

Ross Hartter, University of Missouri, Dr. Sheila Grant, University of Missouri, Dr. Shramik Sengupta, University of Missouri

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Abstract

Traditional plastics are derived from petroleum and are non-biodegradable. Currently, there is a push to reduce the dependence on petroleum derived products and a move towards biodegradable products. Polyhydroxyalkanoates (PHAs) are a group of biodegradable bioplastics that are produced by a wide variety of microorganisms, mainly as a storage intermediate for energy and carbon. PHAs have similar melting point to polypropylene and a Youngs modulus close to polystyrene. There are two major issues restricting the large scale production and utilization of PHAs: 1) the downstream processing of bacterial cultures to extract the valuable PHA and 2) cheap carbon substrate. Secreting PHA from Escherichia coli could potentially reduce downstream processing costs as it will be easier to separate secreted PHA from the biomass. Secreting PHA utilizes the process of type I secretion with hylA signal peptides. Secretion should enable the recombinant bacteria to have increased production of PHAs, and to continue to produce PHA that does not need to be harvested using traditional toxic solvents methods. To help understand secretion of PHAs, the green fluorescent protein (GFP) can be tagged to the PHA polymerase enzyme encoded by phaC. phaC is part of a three gene cassette that includes phaA and phaB and are required for protein expression and ultimately PHA production. In addition to GFP, visualizing the secretion process with SEM and TEM can further enhance the understanding of the secretion phenomena. To address the issue of expensive carbon substrate, cheap alternatives can be used. The wet lipid extraction process (WLEP) uses algae grown on wastewater as a feedstock to produce biodiesel. One of the side streams of the WLEP is the aqueous phase which is rich in simple sugars such as glycerol. Growing the bioplastic producing bacteria on this substrate can potentially make PHA production economically feasible.

IBE Abstract # 451

Nanobead and aptamer based QCM biosensor for rapid detection of avian influenza virus

General Poster Session & Student Poster Session and Competition

Luke Brockman, Ronghui Wang, Jacob Lum, Lisa Kelso, and Yanbin Li, University of Arkansas

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Abstract

There has been a need for rapid detection of Avian Influenza virus (H5N1) due to it being a potential pandemic threat. Most of the current methods, including culture isolation and PCR, are very sensitive but require specialized laboratories and trained personnel in order to complete the tests. Our goal is to design a biosensor that will be able to rapidly detect the H5N1 virus using a quartz crystal microbalance (QCM) and nanobead amplification. Magnetic nanobeads (150 nm in diameter) have a large surface/volume ratio which allows for faster movement and a higher target molecule binding rate which is ideal for its use as a mass amplifier for our target H5N1 virus. Our system also uses aptamers for the capturing of the virus rather than antibodies. Aptamers have unlimited shelf life and are temperature stable which allows them to give much more consistent results compared to antibodies, specifically for in field applications. They also have very similar binding efficiencies which make them a very promising alternative. The surface of the gold electrode was first layered with streptavidin in order to immobilize the biotin labeled aptamers. These aptamers would then capture the target virus, resulting in a frequency change. This change would be further amplified by the addition of aptamer coated nanobeads, which would then bind to the captured virus. This would cause an additional increase, allowing lower concentrations of virus to be detectable. Preliminary results have proven this concept by giving detectable signals for both the capture of virus and amplification with nanobeads, which was also confirmed with scanning electron microscopy.

IBE Abstract # 448

Simulation of micro particle movement and alignment in an electric field

General Poster Session & Student Poster Session and Competition

Yu Zhao, Clemson University; Johnie Hodge, Clemson University; Guigen Zhang, Clemson University, Bioengineering department, Clemson University

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Abstract

Highly X-ray luminescent Eu-doped Gd2O3 nanoparticles are successfully synthesized by a simple wet chemical precipitation method. Influences of dopant concentration, annealing temperature and time in doped nanoparticles have been investigated. The samples are characterized by X-ray diffraction (XRD) and transmission electron microscope (TEM) to investigate the size and structure of the as prepared nanoparticles. In addition, X-ray luminescence of doped nanoparticles has also been investigated. When excited by X-ray, a strong red light is emitted that can be seen by naked eyes. The processing condition dependent luminesce intensity engineering in Gd2O3: Eu was achieved to attain the deliberate color tunability and demonstrated successfully, which are potentially important for life science application.

IBE Abstract # 443

Cellular Responses to Anti-cancer Drug in 3D and 2D Cell Cultures

General Poster Session & Student Poster Session and Competition

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Abstract

Although 2D cell culture is widely used in cell-based biosensors and assays, it has been recognized that 2D cell culture conditional cannot adequately simulate the natural cellular microenvironments in which cells inhabit environments with 3D features. Research has found that cells in 2D culture environment differ physiologically from the cells in 3D culture environment. In this study, we developed 3D cell cultures of oral cancer cells using Matrigel as matrix and investigates the cellular responses to different anti-cancer drug in developed 3D cell cultures in comparison with the traditional 2D cell culture. Cell proliferation rate in 3D and 2D were determined, showing cell proliferation rate in 3D was faster than that of 2D culture. In both 3D and 2D cultures, the dose response ranges and IC 50 of three different anti-cancer drugs with different action mechanisms, including bleomycin, erlotinib, and enzastaurin, were determined and compared.

IBE Abstract # 442

Developing Assembly Methods for Genetic Circuits used to Optimize Metabolic Pathways

General Poster Session & Student Poster Session and Competition

Caleb Carr, Missouri Western State University; David Carr, Missouri Western State University; Eddie Miles, Missouri Western State University; Jerrad Morton, Missouri Western State University; Sachith Polpitaya, Missouri Western State University; Kamay Tru, Missouri Western State University

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Abstract

The overall goal of the project is to engineer a generalizable synthetic biology process that harnesses natural selection in E. coli to optimize the production of any particular metabolite. We have evaluated two methods of DNA assembly (Gibson and Golden Gate) for inserting libraries of parts (promoters, RBS elements, codon optimized alleles, and degradation tags) into a genetic circuit. We have demonstrated assembly of promoter libraries into a reporter construct using both methods. For the Golden Gate assembly approach, we designed a new system by which pairs of type IIs restriction enzymes can be used to repeatedly remove and replace modular internal parts. We will apply our assembly methods to the optimization of a genetic circuit for the conversion of caffeine to theophylline as a proof of concept. Subsequent research will address the scalability of the system to multiple gene circuits controlling complex metabolic pathways. Supported by NSF RUI grants MCB-1120578 to Davidson College and MCB-1120558 to Missouri Western State University.

IBE Abstract # 439

Engineered B-Cell Biosensor for Specific, Sensitive and Rapid Detection of E. coli O157:H7

General Poster Session & Student Poster Session and Competition

Ling Wang, University of Arkansas, Zhejiang University; Ronghui Wang, University of Arkansas; Yanbin Li, University of Arkansas, Zhejiang University; Byung-Whi Kong, University of Arkansas;Kaiming Ye, University of Arkansas; Sha Jin, University of Arkansas

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Abstract

Foodborn pathogens are the major issue in food safety and rapid detection is needed to more effectively identify pathogens in food. The objective of this study was to develop an engineered B cell biosensor with better combined speed and sensitivity than current methods for rapid detection of E. coli O157:H7, one of the major foodborn pathogens. The biosensor was based on the genetically engineered B lymphocyte which was introduced with a pair of Fluorescence Proteins (FPs) and Calcium indicator. When E. coli O157:H7 attached to its specific receptors on the B cell surface, B cell receptors (BCR) induced Ca2+ signal pathway leading to Ca2+ flux. Then Ca2+ flux activated the FRET (fluorescence resonance energy transfer)-based Calcium indicator to report the fluorescence signal change and indicated the presence of E. coli O157:H7. A genetically encoded fluorescent reporter for Ca2+ (TN-XXL) was transfected into the B cell that consisted of fusions of cyan fluorescent protein (CFP), a troponin C-based Calcium indicator and yellow fluorescent protein (YFP). The proof of concept for the developed engineered B cell biosensor was conducted using E. coli O157:H7 at a concentration of 105 cfu mL-1. The fluorescence change of fluorescent proteins induced by FRET was observed.The ongoing research focuses on the optimization of biosensor parameters and then the evaluation of the developed biosensor for detection of E. coli O157:H7 in foods.Keywords: engineered B cell, biosensor, E. coli O157:H7, bacteria detection.

provide biofuels producers with data-driven analysis to minimize environmental impacts and production expenses.

IBE Abstract # 437

Bioenergy Landscape Design to Minimize Cultivation Emissions and Production Expenses

General Poster Session & Student Poster Session and Competition

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Abstract

Biofuels have been promoted as a promising solution to the domestic energy security problem. However, the long-term viability and sustainability of biofuels will hinge on their ability to reduce greenhouse gas (GHG) emissions and costs relative to petroleum-derived gasoline and diesel fuels. Investigation of the full lifecycle of cellulosic biofuel production reveals that feedstock cultivation and transport are highly significant emissions sources, sensitive to the spatial distribution of cropping areas and conversion facility location. Here we introduce a spatially-explicit decision support system (DSS) to identify the bioenergy feedstock landscape design minimizing total GHG emissions and costs associated with biomass production and conveyance at a regional scale. This DSS is built upon the DayCent biogeochemistry model, a tool capable of accurately estimating cultivation impacts for various biofuel feedstocks. The analysis takes into account not only soil GHG emissions, but also the embodied emissions of fertilizers and other farming supplies as well as fuels used for field operations. We then derive a Knapsack problem based upon integer linear programming to describe the optimization objective, and solve it via different approaches including combinatorial, dynamic programming, branch and bound techniques, and a heuristic approach. After considering the runtime versus accuracy trade-off, the heuristic approach is selected to solve the mathematical model. Preliminary results are presented for an illustrative case study of switchgrass production in the SW Kansas region, where of one of the first generation of commercial-scale cellulosic biorefineries in the US is currently under construction. We find that up to 75% of the total feedstock GHG emissions and up to 55% of the total feedstock cost can be reduced by an optimal biofuels landscape design, in comparison to a random assignment of cropping areas and facility locations. This case study supports a larger effort to mobilize this DSS into a user-friendly web-based platform to

IBE Abstract # 433

Using E. coli to Determine Optimal DNA Design for Metabolite Production

General Poster Session & Student Poster Session and Competition

Ben Clarkson, Davidson College; Becca Evans, Davidson College; Betsy Gammon, Davidson College; Meredith Nakano, Davidson College; Caroline Vrana, Davidson College; Caleb Carr, Missouri Western State University; David Carr, Missouri Western State University

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Abstract

A common problem in commercial use of microbes for metabolite production is the evolution of the population away from maximum production of the desired end product. Natural selection favors cells that stop making the desired metabolite so the problem recurs with every production cycle. Synthetic biologists try to guess how to reengineer the desired pathway for optimal production, but they cannot do so efficiently since it is impossible to know all the dynamic responses of cells to an altered metabolism. We wanted to harness natural selection as a mechanism to optimize product formation in E. coli by constructing a series of rewards and punishments. The goal is to provide a mixed population of cells with a variety of reengineered metabolic pathways and have natural selection determine optimal production. We have identified components of the reward and punishment systems for optimized production of theophylline as a proof of concept. All the components that have been tested work as expected. We will report on our progress and outline future steps towards our goal of mutualistic symbiosis between humans and microbes to maximize commercial production of desired metabolites. Supported by NSF RUI grants MCB-1120578 to Davidson College and MCB-1120558 to Missouri Western State University.

Keywords: cost-effective immunosensor; impedance spectroscopy; avian influenza virus; Asian H5N1 field strain

IBE Abstract # 432

Development of a Cost-effective Impedance Immunosensor for Rapid and Specific Screening of Avian Influenza Virus H5N1 Asian Field Strain

General Poster Session & Student Poster Session and Competition

Ronghui Wang, University of Arkansas; Xiaofei Yan, China Agricultural University; Zhanming Li, Zhejiang University; Yuntao Li, Chinese Academy of Sciences; Peirong Jiao, South China Agricultural University; Dong An, China Agricultural University; Mauhua W, University of Arkansas

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Abstract

Avian influenza (AI) H5N1 is still a thread to both poultry and human health. The new field strains of AI H5N1 identified recently presents the need for rapid, specific and low-cost screening methods. The objective of this study was to develop a cost-effective impedance immunosensor for rapid detection of avian influenza virus H5N1, which is affordable for in-field screening tests. A gold interdigitated microelectrode (IDME) (25 pairs of fingers with 10 μm finger width and space) was designed and fabricated with low cost. Three monoclonal anti-H5 antibodies specifically against a recent Asian H5N1 field strain were developed and then evaluated using Dot-Blot. The best one of these antibodies was immobilized on the gold surface of IDME through protein A. The binding of AI viruses onto the antibody-coated microelectrode surface resulted in a change in the impedance, which was measured in a frequency range of 10 Hz to 1 MHz in the presence of 10 mM $[Fe(CN)_6]^{3-/4-}$ as a redox probe. The impedance immunosensor could detect target AI H5N1 virus at a titer higher than 2^1 HAU/50 μl within 1 h. A linear calibration curve for the titer of AI virus and the impedance value was obtained between 2^1 to 2^4 HAU/50 μl. No interference was detected from non-target virus including Newcastle disease virus and AI virus H9N2. Equivalent circuit analysis indicated that the electron transfer resistance was responsible for the impedance change due to the biomaterial immobilization and H5N1 virus binding. Further research will focus on the evaluation of this immunosensor using tracheal and cloacal swabs from infested poultry and the design and construction of a prototype of the instrument for in-field applications.

will be used to identify any contamination and disruption. Once samples are taken and analyzed, the sample sites were plotted on these maps using GIS programming.

In order to correctly interpret the methane results, biogenic and thermogenic methane concentrations must be differentiated. Biogenic methane is biologically produced by anaerobic micro-organisms, whereas thermogenic methane is physically produced from pressure and temperature changes; this correlates to the methane that is extracted by drilling for burning. Overall the groundwater quality of the samples was pretty good. Only two wells had methane concentration above 7ppm. Some wells had “very hard” water. A few wells had high Manganese and high Chloride concentration; however, these contaminants only have a recommended maximum contaminant level, so aren’t dangerous. No statistical relationship between methane concentration or fractionation and distance gas well or topographic position was found. Currently, we are doing spatial analysis of methane patterns by the depth of water well and substrate that well taps into (either bedrock or unconsolidated till). We are now mapping the dissolved solids for presentation on website and doing further spatial autocorrelation. After further study of the groundwater after the drilling process, the C-13 ratio can be compared to again. If the differential is less negative, it can be concluded that the methane is becoming more thermogenic; therefore, the drilling/ fracturing process has an effect on the groundwater.

IBE Abstract # 428

Baseline evaluation of groundwater quality in central New York in the face of shale gas development

General Poster Session & Student Poster Session and Competition

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Abstract

Due to the controversy surrounding Natural gas drilling in New York State, it is important to assess the validity of claims that groundwater methane concentration is increased with the advancement of well drilling. Hydraulic fracturing is an efficient way to extract natural gas from tight shales and has been practiced for decades; however, recent media has brought attention to its potential concerns. A potentially toxic "fracking fluid" is injected at high volume and velocity to open up cracks in the shale; however the depth that this fluid is injected is far deeper than drinking water aquifers. There is still uncertainly as to whether methane contamination is a result of the fracturing process (FracFocus Chemical Disclosure). Osborn et al found a correlation between methane concentrations in groundwater samples to the distance that the sample was taken from active gas wells; however this study is criticized for being biased and lacking in baseline data.

The study done by the Cornell Soil and Water lab obtained a baseline assessment of water quality prior to hydraulic fracturing in NY. Further, we were interested in assessing water quality near existing conventional gas wells, in order to make some preliminary comparisons.

In Chenango and Broome County, NY, 147 groundwater samples were obtained. The sample locations were randomly selected. Ion chromatography, gas chromatography, and Trace gas Isotope Ratio Mass Spectrometer were used to analyze samples. Further, samples were assessed for total suspended solids, specific conductivity, and pH because these are generally used indicators for water quality. These values

IBE Abstract # 424

Physiological Methods for Maximal Fatty Acid Production in Genetically Engineered Cyanobacteria

General Poster Session & Student Poster Session and Competition

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Abstract

Photosynthetic microorganisms such as cyanobacteria and green-algae are an attractive prospect for the production of sustainable bioproducts and biofuels for many reasons, including metabolic efficiency and genetic amenability. Strains of the cyanobacterium Synechococcus sp. PCC 7002 have been genetically modified to produce and excrete medium-chain fatty acids suitable for use as fuel precursors. It has been shown that in green algae, physiological conditions such as nutrient limitation can cause significant changes in metabolism and carbon storage (Work et al., 2011). The fatty-acid secreting cyanobacteria mutants had not been studied in this way. In an effort to both understand the mutant metabolism and increase their FFA production, the effects of various physiological conditions on cyanobacterial growth and free fatty acid (FFA) production were assessed. Nutrient limitation had only neutral or negative effects on growth and productivity, unlike previous findings in algal systems. In contrast, both highlight intensity and urea utilization treatments showed increased FFA production, which did not directly correspond to cell density. Studies in Synechococcus have linked lipid peroxidation to high intensity light and, more unexpectedly, urease activity (Maeda et al., 2008)(Sakamoto, 1998). Thus, the trends in FFA production may be related to the effects of lipid peroxidation on the mutants, and they support the proposed mechanism of the mutants FFA-secretion involving impaired membrane lipid-recycling.

IBE Abstract # 558

Recognization of Poly(dimethylsiloxane) using phage displayed peptides

General Poster Session & Student Poster Session and Competition

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Abstract

The development of a general approach for the non-destructive chemical and biological functionalization of poly(dimethylsiloxane) (PDMS) could expand opportunities for using PDMS in both fundamental studies and a variety of device platforms. PDMS is a silicon elastomer which shows unique optical, electrical, mechanical, and chemical and biological compatibility. Phage display has emerged as a powerful method for selecting peptides that possess enhanced selectivity and binding affinity toward a variety of targets. In this report, we demonstrate for the first time a powerful yet benign approach for identification of binding motifs to PDMS via comprehensively screened phage displayed peptides. Our results show that PDMS can be selectively recognized with peptide-displaying phages and bifunctional peptides. Further, along with the development of PDMS-based microstructures; recognition of PDMS with phage displayed peptides can be specifically localized in these microstructures. We anticipate that these results could open exciting opportunities in the use of peptide-recognized PDMS in fundamental biochemical recognition studies, as well as applications ranging from analytical devices, hybrid materials, surface and interface, to cell biology.

there were 3 out of 7 virulence-related genes showed significant decrease on expression levels (>2 fold decrease).

IBE Abstract # 419

Coupling of single-walled carbon nanotubes with near-infrared radiation inactivates Bacillus anthracis spores and stimulates spore germinations

General Poster Session & Student Poster Session and Competition

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Abstract

B. anthracis is a pathogen that causes life-threatening disease--anthrax. The bioterrorism attack involving anthrax spores in 2001 brought acute public attention and caused widespread anxiety. B. anthracis spore is the dormant form of the bacterial cell which is highly resistant to extreme temperatures and harsh chemicals. Research has demonstrated that single walled carbon nanotubes (SWCNTs) exhibited strong antimicrobial reagents to bacterial vegetative cells. But due to the protective structure of spore, B. anthrcis spores are resistant to SWCNT treatment. The objectives of this study were to demonstrate if the coupling of SWCNTs with near-infrared radiation (NIR) can effectively inactivate B.anthracis spores and affect spore germinations. We tested both the effects of SWCNTs alone and the coupling of SWCNTs-NIR on the activities of B. anthracis Sterne 34 spores. The results showed that the treatment of 10 Âµg/mL SWCNTs coupled with 20 min NIR significantly improved the antimicrobial effect by doubling the percentage of viable spore number reduction compared with SWCNTs alone treatment (88.17% vs. 41.70%). At the same time, SWCNTs-NIR treatment activated the germination of surviving spores and their dipicolinic acid (DPA) release. The results suggested the dual effects of SWCNTs-NIR treatment on B. anthracis spores: enhanced the sporicidal effect and stimulated the germination of surviving spores. To study if the treatments changed the expression levels of germination-, virulence-, and regulation-related genes, real-time PCR was performed. The results demonstrated that SWCNTs-NIR increased expression levels (>2-fold) in 3 out of 6 germination-related genes tested in this study, which was correlated to the activated germination and DPA release. SWCNTs-NIR treatment either induced or inhibited the expression of 3 regulatory genes detected in this study. When the NIR treatment time was 5 or 25 min,

IBE Abstract # 413

X-ray excited luminescence properties and applications of Gd2O3: Eu nanophoshors

General Poster Session & Student Poster Session and Competition

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Abstract

Highly X-ray luminescent Eu-doped Gd2O3 nanoparticles are successfully synthesized by a simple wet chemical precipitation method. Influences of dopant concentration, annealing temperature and time in doped nanoparticles have been investigated. The samples are characterized by X-ray diffraction (XRD) and transmission electron microscope (TEM) to investigate the size and structure of the as prepared nanoparticles. In addition, X-ray luminescence of doped nanoparticles has also been investigated. When excited by X-ray, a strong red light is emitted that can be seen by naked eyes. The processing condition dependent luminesce intensity engineering in Gd2O3: Eu was achieved to attain the deliberate color tunability and demonstrated successfully, which are potentially important for life science application.